A MANUAL

OF

DISSECTION AND HISTOLOGY

FOR THE USE OF

CLASSES IN PHYSIOLOGY

IN

HIGH SCHOOLS, NORMAL SCHOOLS, AND ACADEMIES

BY

G. H. FRENCH, A.M.

PROFESSOR OF PHYSIOLOGY AND BIOLOGY AND CURATOR IN THE SOUTHERN ILLINOIS NORMAL UNIVERSITY

MEMBER OF THE ENTOMOLOGICAL SOCIETY OF FRANCE, OF THE ENTOMOLOGICAL SOCIETY OF BELGIUM; ASSOCIATE MEMBER OF THE NATURAL HISTORY SOCIETY OF LUBECK, OF THE ENTOMOLOGICAL SOCIETY OF ONTARIO, OF THE ENTOMOLOGICAL SOCIETY OF NEW YORK, OF THE AMERICAN ENTOMOLOGICAL SOCIETY, AND OF THE PHILADELPHIA ACADEMY OF SCIENCES.

PHILADELPHIA

J. B. LIPPINCOTT COMPANY

PREFATORY NOTE.

DOUBTLESS other teachers of physiology besides the writer have felt the need of a brief laboratory manual of dissection and histology, and it is to supply such a need that this little volume is prepared. The plan of the manual is for the pupils to prepare their microscopical sections before dissecting any, if it is desired to make permanent mounts. A good way is to have a bird and a cat or a rabbit; divide the class into groups of as many as work together, and give each division of the class some part of the bird or cat or both of which to prepare sections. These may be tagged and all put into the same jar of hardening fluid, if only one is used, and some pupils may be detailed to make the daily changes till the time comes for embedding and cutting. Let each division embed the part given it, putting the number of tag on the block, the teacher keeping a list of the parts

given out to the divisions. When ready for cutting, let each division make sections of their parts ready for mounting, and then each pupil mount for himself a section of the prepared part. This will give all the pupils sections of each organ or part to be studied, and give all a chance in preparing the sections. When the sections of the different parts are mounted, they may be studied with the microscope in connection with the dissection of the part. This need not prevent the studying of fresh material histologically if desired by the teacher.

After pupils have become familiar with a simple method of preparing sections, then they may be allowed to do the work of some more complicated scheme.

Each teacher must be his own judge of the amount of this kind of work for a class to do. It will be found better, however, to study an organ or a part in the laboratory after the class has studied that organ or part in the text-book. Birds are always accessible at any season of the year, hence I have placed the study of a bird first. The bird as a specimen for study may be used through the whole series of parts, or a

mammal or amphibian may be used first or alternated with the bird. Some benefit may be found in comparing the parts of all three. It will be a benefit, also, to have the pupils see how they all differ in their structure from man.

I have given throughout the celloidin process of embedding and cutting because it is simple and managed with but little apparatus. For this reason it seems well adapted for high school and normal school work.

The questions and explanations in Part I. are not exhaustive, but are such as the average pupil who studies physiology should be able to answer or understand. The writer has not included all the organs, as the limited time devoted to physiology in high schools or normals will not permit very extensive laboratory work.

The writer has figured only two pieces of apparatus to be used, the microscope and the microtome. These are illustrated because it seemed best to make some explanation of these. For other apparatus the teacher is referred to the large and well illustrated catalogues of microscopic materials, such as is published by the Bausch & Lomb Optical Company, of Rochester,

and others. Much or little apparatus can be used in this work according to the means or inclination of the teacher.

Electrotypes for illustrating this volume have been obtained from the following sources:

From the Bausch & Lomb Optical Company, Rochester, New York, Figs. 1, 2.

From P. Blakiston, Son & Co., Philadelphia, taken from Stirling's "Outlines of Practical Histology," Figs. 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23, 24, 25, 26.

Fig. 19 is new, and was made from a cross-section of the glandular stomach of a meadow-lark by the St. Louis Engraving and Electrotyping Company.

EXPLANATION OF TERMS.

Ascinus, a group of cells forming a gland unit, as a lobule or a tubule.

Attachment, end of a muscle whose support moves when the muscle contracts.

Capsule, covering of lens, kidney, spleen, etc.

Condyle, an articulating surface.

Decalcified, the lime taken out of a bone.

Endocardium, membrane lining the heart.

(H.), high power of the microscope.

Histological, studied by the aid of the microscope.

(L.), low power of the microscope.

L. S., longitudinal section.

Origin, the end of a muscle whose support is not moved when the muscle contracts.

Pectoral Girdle, the bony ring in a frog to which the front limbs are attached.

Pelvic Girdle, the bony ring in a frog to which the hind limbs are attached.

Periosteum, the membrane round the bone.

Trabeculæ, connective tissue from the capsule of the spleen or other similar organ that extends into the organ from the inner part of the capsule.

T. S., transverse section.

V. S., vertical section.

PART I.

DISSECTION.

A MANUAL

OF

DISSECTION AND HISTOLOGY.

DISSECTION.

The object of dissection is to separate the various parts of an animal in such a manner as to show their shape, size, and relation to each other. To do this it is necessary to sever the connective tissues that bind the parts together; but this should be done in a careful and precise manner. At the beginning the student should learn that promiscuous hacking specimens to pieces is not dissection. Credit should not be given for such work.

It is well to have a few general rules that should be observed in dissection, such as the following:

1. Fix the specimen in whatever position will be the most convenient for work. If a dissecting-pan be used, it will probably be best to fasten the specimen with the head from the operator.

2. Moisten the tissues occasionally with water or normal salt solution to prevent drying.

3. In dissecting muscles, nerves, and blood-vessels, stretch them slightly and work in the direction of their length.

4. Do not allow scraps to accumulate on the specimen. Sponge away clots of blood.

5. Do not try to work with dull instruments, as you cannot do good work with them. Therefore keep scalpels sharp and clean.

6. When through with the work for the day, thoroughly clean all instruments that have been used. Do not wipe at first with the chamois-skin, but wipe first with a damp cloth, then with a dry one, and last with the chamois-skin.

NOTES AND DRAWINGS.

Full notes and drawings should be made of every dissection and of all the parts studied histologically. Besides answers to the questions given, descriptions may be made of other parts observed. Make notes and drawings at the time the work is done, and leave them in the place assigned by the teacher.

A set of Boyer's "Blanks for Laboratory

Notes and Drawings" will be the best and cheapest good material that can be had for notes and drawings. Each set consists of a block of writing-paper, a block of drawing-paper, two covers for the work after it is done, and a set of brass fasteners to bind all together in a neat book.

All drawings should be made from the objects studied and not from some picture, either in the manual or found elsewhere. The teacher should not give credit for a drawing that is not made from the object studied.

If the drawings are made from the microscope, they can be made in a circle, two and one-half to three inches in diameter, and the drawing include what is seen of the object in the field without moving the slide. The drawing should be accompanied by the name of the object and the amount of magnification. The latter may be expressed as follows: "$\times$ 96" for magnified ninety-six diameters.

DISSECTION OF A BIRD.

MUSCLES.

Skin the bird carefully without tearing or removing any of the muscles. Great care must

be taken with the wings, or one muscle will be removed with the skin.

1. Find a double muscle on the anterior side of the humerus, united at the shoulder but disconnected at its insertion or attachment, one of its long tendons being inserted at the elbow and the other at the wrist,—the tensor plicæ alaris. Pull gently with the forceps. Attachment? Use? Find below this the brachialis biceps. Origin? Attachment? Pull with the forceps. Use? On the back side of the humerus find the brachialis triceps. Origin? Attachment? Use? Draw.

2. Find the pectoralis major, or large breast muscle. Its origin? Attachment? Pull with the forceps. Result? From this what is its use? Separate this muscle from its origin and turn it up. Find under this the pectoralis minor, or second breast muscle. Origin? Trace to its attachment. Pull with the forceps. Use? Do these two muscles have the same use? Birds have a third breast muscle, whose origin is at the anterior end of the sternum, and which is attached in the upper outer end of the humerus, which it helps to raise. Find this. We do not

have this muscle. It answers in part to our subclavius.

3. On the back of the shoulder find the trapezius, whose origin is the last cervical and the first dorsal vertebræ. Attachment? Pull with the forceps. Use? Find beneath this the rhomboideus. Pull with the forceps. Origin? Attachment? Use? On the outer part of the shoulder find the deltoideus muscle. Pull with the forceps. Use? Besides these there are several small muscles on the back part of the shoulder of the bird.

4. On the leg find the sartorius, extending from the outer part of the os innominata to the inner side of the knee. Pull gently with the forceps. Use? Which end is the origin and which the attachment? Remove this and a muscle on the outside of the leg, the tensor vaginæ, and the main muscles that move the leg back and forth may be seen. In front find the rectus femoris, inside this the vastus internus and the crureus, the inner bundle being made up of two muscles, and on the outside the vastus externus. Find these four. Use? Draw.

5. On the back of the limb are the gluteal

muscles,—the gluteus externus, the gluteus medius, and the gluteus minimus, in this order from the outside. Sometimes the last is absent. Find these. Use? These are the main muscles on this part of the leg, and are the same muscles that are to be found on this part of our lower extremities. Below the knee on the back side are two muscles, the gastrocnemius and the soleus, the latter beneath the first. Find and pull with the forceps. Origin? Attachment? Use? The tendon is the tendo Achillis.

6. (Histological.) (L. and H.) Make a drawing of as much as you can see of the muscle, giving all the markings you can see. What is the shape of the fibres? Do you find a sarcolemma? With a transverse section see the ends of the fibres. Shape? Draw. If you have a section double-stained with hæmatoxylin and borax-carmine, see if you can find nuclei to the fibres and the cross-striæ. If you can, draw. For explanation of cross-striæ, see Fig. 9 in Part II.

BONES.

1. Remove the tissues sufficiently to find the bones readily. Find the humerus, ulna, and

radius. How many of each in one wing? What kind of a joint is the shoulder-joint? What kind at the lower end of the humerus? Which is the larger, the ulna or the radius? Make out as many bones of the hand and wrist as you can. Draw.

2. Find the femur and tibia. Is there a fibula? If so, how far below the knee does it extend? See if you can find a patella. The bone in the scaled portion of the leg is regarded as a combination of the tarsal and metatarsal bones, and is called the tarso-metatarsus. To this the toes or phalanges are attached below. Draw.

3. On the head find the occipital bone. By how many occipital condyles or joints is this articulated to the first cervical vertebra? Find the foramen magnum, or passage for the spinal cord. Shape? On each side of the foramen magnum is a ridge, the paroccipital ridge. In some birds, as the ostrich, ducks, etc., the occipital bone is formed of four bones,—these ridges being parts of two of them,—the occipital, the supraoccipital, and the two paroccipitals. How is it with your bird? See if you can find the

temporal and frontal bones. How many frontals? Draw.

4. The upper mandible is a bone encased in horn, the premaxillary, and it is joined to the cranium by a movable joint. The lower mandible is also a bone sheathed in horn, answering in part to our inferior maxillary. It is joined to the cranium by a separate bone, the quadrate bone. There is another slender bone extending from about the middle of this to the base of the premaxillary, the maxillo-jugular bar. This serves to raise the upper mandible when the lower mandible is lowered. Find all these and draw. How many of these have we?

5. How many cervical vertebræ are there? How many dorsal? The dorsal are those to which the ribs are attached. Are the dorsal vertebræ movable or immovable? Find the os innominata. Is it of the same shape as ours? To what extent is it attached to the vertebræ? Shape of the sternum? How many of the ribs are attached to the sternum? How many bones form the shoulder? To what is the wish-bone analogous in our skeleton?

6. (Histological.) Draw and locate all the

canals and openings you can find in your section. Find the periosteum. If you have a section from the end of a bone, find the interarticular cartilage. Are there any cells in this? Are there any nuclei? For explanation of these, see Fig. 10 in Part II.

DIGESTIVE ORGANS.

1. Has the bird teeth? How do the mandibles differ from our jaws? Use of the mandibles? Trace the œsophagus to the crop. Use of the crop? Have we anything analogous to the crop? What is in it?

2. Trace from the crop to the glandular stomach, the enlarged part just before the gizzard. This supplies the gastric juice. How does it differ from the gizzard? How does it differ from the œsophagus?

3. What is the shape of the gizzard? Cut it open. What is in the inside? Find three coats. What are they? Use of each? Draw the crop and gizzard and the included part.

4. Find below the gizzard the small intestine. Trace to two branches that are given off. These are the cæca.* Below or beyond the

* The cæca are not present in all birds, as woodpeckers.

cæca is the large intestine. How does it differ from the small intestine? Is there any opening to the outer end of the cæca?

5. Find the pancreas. Where is it? Where does its duct empty? Shape? Find the liver. Is there a gall-bladder? Where does the biliary duct empty? What holds all these organs in place in the abdomen?

6. (Histological.) **Glandular Stomach.**—T. S. (L. and H.) Beginning with the outside, locate the peritoneum, the transverse and longitudinal muscular coats, and a layer of connective tissue next to the glands, or the submucous coat. Find next a series of plumose glands, and next to these a series of straight tubular glands. Where does the duct of the plumose gland empty? These glands are peptic, or secrete the gastric juice. Draw, locating all these parts. See explanation and Fig. 19 in Part II.

7. **Gizzard.**—Distinguish the outer or serous coat, the muscular coat, and the inner or fibrous coat. Which is the thickest? Why? Are there any glands in the inner coat? (You can tell partly by the difference in staining.) Draw a section.

8. **Small Intestine.**—Find on the outside the first three coats, the same as for the glandular stomach, the serous, transverse muscular, and longitudinal muscular coats. Inside these find the glands of Lieberkühn, and inside these the villi. Draw, and in the drawing distinguish these. Are there any Peyer's glands?

9. **Liver.**—Find the little openings, the intralobular veins. See if you can make out the boundaries of the lobules. Find the outer serous membrane or the capsule. Draw. How does your section compare with Fig. 14 in Part II.?

RESPIRATION.

1. Where are the nostrils located? Pass a pin into one of them and see where the inner opening is. Find the glottis. What is its shape? Which way do the papillæ point? Use of the papillæ? Do we have papillæ round the glottis? Is there an epiglottis? Draw.

2. Are the rings of the trachea entire or not? How do they differ from ours? At the bifurcation of the trachea find the true vocal cords. See if you can find a membrane extending upward, free at the upper end, inside the tra-

chea. This is the "trilling" organ of the bird. Draw.

3. How many lungs are there? Are they lobed like ours? Color? Is there a diaphragm? How far back do the lungs extend? Into what part of the lungs do the bronchi enter? Draw in position. Remove. Does the pleura cover both sides of the lungs?

4. (Histological.) (L. and H.) **Trachea.**—Distinguish three coats, the inner or mucous, the middle or cartilaginous, and the outer or fibro-muscular. What kind of epithelium lines the inner? If your section shows the rings, see that the rings are continuous. A longitudinal section shows the cut ends of the rings. Shape of ends? Draw.

5. **Lungs.**—Make out as many of the tissues as you can. Are the air-cells smooth, or can you see depressions in them? Find the pleura. See if you can find any ciliated epithelium in the larger air-passages. Draw.

CIRCULATION.

1. Find the heart. How far out on each side does the pericardium extend? Open the peri-

cardium and remove it. On the right side find coming from below the inferior vena cava, the auricle being slightly enlarged where it empties into a sinus venosus that is more prominent in frogs. There are two superior venæ cavæ, the right and left, as in frogs. The blood is returned to the left auricle by two pulmonary veins, one from each lung. How does this differ from ours?

2. The pulmonary artery arises from the right ventricle and divides to go to each lung. The left ventricle sends out the aorta that divides into three arteries. How does this differ from ours? Draw. Open the heart and find the auricles and ventricles. Note carefully the valves between the auricles and ventricles on both sides and also the tendinous cords. Are they the same on both sides? How do they differ from ours? Draw.

3. From which branch of the aorta does the descending aorta come? Find the portal vein, the hepatic vein, and the two thoracic ducts. In birds there are two of these instead of one. In the hollows of the pelvis find the dark-red kidneys. Shape?

4. (Histological). **Heart.**—L. S. (L. and H.) Of what kind of muscular tissue is the heart composed? Is there a sarcolemma? (See a double-stained section for this.) Find the pericardium, the endocardium. Draw. Are there any transverse striæ?

5. **Kidney.**—Find the tubules and the Malphigian bodies. Shape of both? The last are the excretory parts of the kidney. With a double-stained section see the individual cells. In what part are the nuclei? How do they compare with Fig. 13 in Part II.? Draw from your section.

NERVOUS SYSTEM.

1. Carefully cut away the upper or outer cranial bones. Find the cerebrum and cerebellum. Is the cerebrum convoluted? Is the cerebellum? How do they compare in size? Find the olfactory lobes. Find back of the cerebrum on each side the optic lobes, a little to one side and between the cerebrum and cerebellum. Find back of the cerebellum the medulla. Draw and locate all these parts before removing the brain.

2. With the point of the scalpel raise the

anterior part of the brain and notice fibres connecting the brain with the skull. These are the cranial nerves. Make a section into the brain through the longitudinal fissure. Notice that there are several openings on the inside of the brain. These are the ventricles of the brain, of which the first is in the olfactory lobes, and the fourth is in the cerebellum. Does the longitudinal sinus extend through the brain? In the cerebellum find the "tree of life." How many branches do you find? Are the branches simple or branched?

3. Is the gray matter of the medulla on the outside or the inside? How does the medulla compare in size with the spinal cord? In order to see the spinal cord well, cut away the neural arches of several of the cervical vertebræ. What is the shape of the spinal cord? Size? Is there a dorsal fissure or sinus? See if there is a ventral sinus.

4. Remove the kidneys from their cavity in the sacrum and find several nerve-threads pass out from the spinal cord and unite to form the sciatic nerve. See if you can find any other spinal nerves.

5. (Histological.) **Spinal Cord.**—T. S. (L. and H.) Find in the centre the gray matter. Shape? In mammals there is a blood-vessel in the centre. Is there in the section you have? See if you can distinguish the separate nerves. Draw.

6. **Brain.**—Find the coat adherent to the brain, the pia mater. How thick is the gray matter? See if you can see the separate nerve-cells. Draw. If you have a properly stained nerve, distinguish the myelin and the axis-cylinder. Draw. For a nerve-fibre, see Fig. 23 in Part II.

SPECIAL SENSES.

1. **Tongue.**—Are there any papillæ on the tongue? If so, where? To what part of the tongue is the hyoid bone attached? Shape?

2. (Histological.) Distinguish, if you can, the different kinds of tissues of the tongue. Is the tip of it covered with skin? If not, what is the tissue? Can you find any taste buds or pits? For the shape of taste buds, see Fig. 25, Part II. Draw.

3. **Eye.**—How many lids are there? Cut away the upper lid and find the muscles by which the eyeball is moved. How many do

you find? Take the eye out. Shape? Draw. Where is the optic nerve?

4. With a sharp scalpel or razor cut through the equator of the eye. In the posterior half you may find a thin lining membrane that you can strip away with the forceps, the hyaloid membrane. The front part of this forms the suspensory ligament that supports the lens. Find its attachment round the outer edge of the iris. Besides this the lens has its own covering, the capsule. Find where the optic nerve enters the eye. Before trying to strip out the hyaloid membrane, see if you can find a membrane that extends from this membrane to or towards the lens, the marsupium of the eye. We do not have this. Use of the marsupium?

5. Take the lens out. Shape? Lay it on a piece of paper having print on it and see that it magnifies. The iris lays on the lens. Is it attached to it? Color of the iris? The cavity between the cornea and the iris is filled with the aqueous humor, the posterior part of the eye is filled with the vitreous humor. How does the cornea differ from the sclerotica? Draw.

6. (Histological.) (L. and H.) In a section showing the front of the eye find the cornea, and outside this a thin membrane, the conjunctiva, or mucous membrane of the eye. See if you can find also a thinner membrane on the inside of the cornea, the endothelium layer. Draw. In front of the lens find the iris. See that the back part of this is formed of the back part of the choroidea. Is the iris thickest at the outer or inner part? See that the choroid part of the iris at the outer part is laid in folds. This is the inner portion of the ciliary process. See outside of this the ciliary muscles between the ciliary process and the sclerotica. See if you can find the two sets of these muscles. These are the muscles of accommodation. Draw.

7. With the section of the back part of the eye find the three coats,—sclerotica, chordea, and retina. If your section shows the optic nerve, which of these coats are absent here? Draw this portion. How many layers or coats can you make out in the retina? See if you can distinguish the nerve-cells and nerve-fibres. Find that the outer layer of the retina is a color or pigment layer, but is separate from the

choroid coat. This is the color layer for the rods and cones. See if you can find the hyaloid membrane. If your section has the marsupium. Shape? Draw.

8. **Ear.**—How many parts of the external ear do you find? Name them. With the bone-saw cut into the cranium so as to see the middle and internal ears. Find the membrana tympani and the chain of bones. Draw.

DISSECTION OF A MAMMAL.

A cat, rat, or rabbit will answer very well for this. It is better not to have the animal too large nor too small. Skin the specimen without tearing the muscles or breaking the bones.

MUSCLES.

1. In front of the humerus find the brachialis biceps. Pull with the forceps. Which end is the attachment? Which end is the origin? On the back of the humerus find the brachialis triceps. Pull. Use? There are other small muscles on this part of the arm, but these are the main opposing muscles.

2. On the breast find the pectoralis major, or large breast muscle. Pull with the forceps.

Origin? Attachment? Use? Under this find the pectoralis minor, or small breast muscle. Origin? Attachment? Use? On the dorsal side of the shoulder find, extending from the vertebræ to the shoulder, the trapezius. Shape? Beneath this find the rhomboideus. Use of these two muscles? These are the four main muscles of the shoulder.

3. On the dorsal side of the forearm are a number of small muscles that are extensors of the toes or phalanges. On the opposite side are the flexors of the toes. Pull each to see its use.

4. On the middle of the abdomen find two long slender muscles, the rectus abdominis, that run from the pubic portion of the os innominata to the ribs. On each side of these two muscles are three muscles that form the abdominal wall, —the external oblique, the internal oblique, and the transversalis. These lie one over the other in the order given, but their fibres run in different directions. Find on one side these four muscles. Back of the trapezius, on the dorsum, find a large sheet of muscle whose origin is in the vertebræ from the fifth dorsal to the fourth lumbar, the latissimus dorsi. Attachment? Use?

5. On the outside of the hip find, first, a long slender muscle that extends from the os innominata to the knee, the sartorius, back of this a broader sheet, the tensor vaginæ femoris. Back of the last are three muscles from the middle of the thigh,—biceps femoris, semimembranosus, and semitendinosus. These five are the outer muscles of this part of the leg. Find them. Draw.

6. Remove the tensor vaginæ femoris and the biceps femoris. There are four gluteal muscles that have their origin mostly in the os innominata but are inserted into the great trochanter or bend of the femur. Find them. There are several other small muscles in this region. On the front of the femur find the rectus femoris. On the outside of this is another muscle, the vastus externus, and on the inside of the rectus femoris are two muscles, the vastus internus and the crureus. Find these four. Attachment? Use?

7. Below the knee, on the under side, find two muscles, one over the other, fixed to one tendon below. The outer is the gastrocnemius, the inner is the soleus. Origin? Attachment?

Pull with the forceps. Use? The tendon is the tendo Achillis, the largest tendon in the body. On the anterior side of the tibia are several muscles, the first large one being the tibialis anticus. Find. Pull these anterior muscles. Use?

8. (Histological.) (L. and H.) With the low power make a drawing of as much of the muscle as you can see. If there is any connective tissue between the fibres indicate it. What is the shape of the fibres? With a transverse section see the ends of the fibres. Shape? With a double-stained section find a fibre that shows the transverse striæ and, if possible, the nuclei. Draw, using the high power. If you find the nuclei, where are they? Shape? Transverse striæ and nuclei are shown in Fig. 9, Part II.

BONES.

1. Remove the tissues enough to find the bones readily. Find the humerus, ulna, radius, carpus, metacarpus, and phalanges. How many of each in one leg? What kind of a joint is the shoulder? To what bones is the clavicle attached? Draw the bones of the fore limb.

2. Find the femur, tibia, fibula, tarsus, metatarsus, and phalanges. How many of each in one hind leg? What kind of a joint is the hip? Is there a patella? What kind of a joint is the knee? Shape of the os innominata? Draw the bones of the lower or hind limb.

3. How many cervical vertebræ are there? How many dorsal? How many lumbar? (The dorsal are the ones to which the ribs are attached.) How many caudal vertebræ? Have we anything that corresponds to the caudal vertebræ of a cat or a rat? If so, what? Is there a sternum?

4. Find the occipital bone. By how many condyles or joints is it articulated with the first cervical vertebra? Shape? Find the parietal bones. Shape? How many frontal bones are there? Find the malar, nasal, vomer, superior maxillary, and inferior maxillary bones. In front of the superior maxillary bones are two small bones, the premaxillaries. We do not have these. How many of each kind of teeth are there? Use of the incisors? Use of the molars?

5. (Histological.) (L. and H.) With the low

power find the periosteum. What connection has this with the bone? If there is a marrow, how does it differ from the bone? Find with (H.) the canals in the bone. Does the bone stain differently from the other tissues? If you have a section showing the end of a bone, see if there is any interarticular cartilage. Has it cells? Draw. For the structure of the end of bone see Fig. 10 in Part II. If you have longitudinal section of two vertebræ, draw them.

DIGESTIVE ORGANS.

1. Is the tongue rough or smooth? If rough, which way do the papillæ point? Where are they? How many openings are there into the pharynx? Name them. Is there a crop in the œsophagus?

2. Shape of the stomach? Draw. On which side of the diaphragm is the stomach? Into which end is the œsophagus inserted? Can you detect any constriction at the pylorus?

3. Where is the liver? Is there a gall-bladder? How many lobes to the liver? Find the pancreas. Shape? Trace the pancreatic and biliary ducts to the small intestine. How far

below the stomach do they join the intestine? Color of the liver? Color of the pancreas? Where is the spleen? Draw.

4. Trace the small intestine to the large intestine. How are they joined? Difference in size? Is there a vermiform appendage? What holds the intestines in place?

5. (Histological.) (L. and H.) **Stomach.**—There are four coats to the stomach,—serous, muscular, submucous, and mucous. For these coats, see Fig. 17, Part II. Find. Shape of the peptic glands, or the glands that secrete the gastric juice? If you have a section showing the glands of both ends of the stomach, see if the glands are of the same shape. See with (H.) if you can make out the cells in the glands. Draw all these parts. Study the structures of Figs. 17 and 18 in Part. II.

6. **Intestines.**—Find the same coats as are to be found in the walls of the stomach. The glands of Lieberkühn and the villi seem to be continuous, the latter internal. Are there any Peyer's glands? (For place and shape of these, see Fig. 20, Part II.) If so, in what part of the intestine? Shape of villi? Draw.

7. **Liver.**—Make out the serous coat or capsule of the liver. The small openings are the intralobular veins. Find. Can you find the boundaries of the lobules? Draw.

8. **Spleen.**—Find the outside layer or capsule. See how many layers or coats you can find in this. Find where the inner layer sends fibres into the interior, or trabeculæ, as they are called. In the interior, in the spleen pulp, make out the blood-vessels and the round Malpighian bodies. With (H.) see if you can distinguish the blood-corpuscles from the leucosites or the white corpuscles. Distinguish these from the fibre-cells. If your section is double-stained, find the nuclei of the cells and draw. See if you can see the capillaries leading to the Malpighian bodies. For explanation of the parts of the spleen, see Fig. 16, Part II.

RESPIRATION.

1. At the base of the tongue find the glottis, epiglottis, and vocal cords. Shape of each. Draw.

2. Are the rings of the trachea entire? If not, on which side is there no cartilage? How do they compare with ours? Draw.

3. How many lungs are there? How many lobes to each lung? Color? Is there a diaphragm? What part of the lung is free?

4. (Histological.) (L. and H.) **Trachea.**—Distinguish the different coats,—mucous, cartilage, and fibro-muscular. Find, if you can, on the inner coat the ciliated epithelium. Draw.

5. **Lungs.**—Find the pleura. Distinguish, if you can, the air-cells, the walls of the larger air-passages, if you have a section that shows these, and blood-vessels. Can you find ciliated epithelium in the larger air-passages? Draw. See Fig. 22, Part II.

CIRCULATION.

1. Find the pericardium. In the heart make out the cavities: the right and left auricles and the right and left ventricles. Are the valves in the two sides alike? Of what kind of tissue are they composed? Find the aorta and pulmonary artery.

2. Trace the pulmonary artery to the lungs. Where does it divide? Find the first artery given off from the aorta, the one that goes to the heart. It is back of one of the semilunar valves. How far from the valve? Is there more

than one? Find the carotid arteries that go to the head. Where are they given off? See if you can find the arteries given off to the stomach and kidneys and the divisions of the aorta in the pelvis.

3. Find the portal vein, the hepatic vein, inferior vena cava, the superior vena cava, jugular veins, and thoracic duct. Where are these?

4. (Histological.) (L. and H.) **Heart.**—Of what kind of muscular tissue is the heart composed? Is it striated? Is there a sarcolemma? (It will take a double-stained section for the last two questions.) Find the endocardium. The outside layer of connective tissue is a continuation of the pericardium. Find this. Draw.

5. **Kidney.**—In a cross-section of the kidney find the tubules and the Malpighian bodies. The last are the real excretory parts of the kidney. With a double-stained section find the cells of which the tubules are composed and the nuclei. Draw. Compare with Fig. 13, Part II.

NERVOUS SYSTEM.

1. Remove the cranial bones that cover the outside of the brain. Is the cerebrum convo-

luted? Is the cerebellum? How do they compare in size? Shape of each? Draw.

2. Raise the anterior part of the brain carefully to find the olfactory lobes. See, as you raise the brain, that there are several fibres extending down into the lower part of the cranium. These are the cranial nerves. One large white one you may recognize as the optic nerve. See how many of these nerves you can find.

3. Make a section into the cerebrum. Where is the gray matter? Where is the white? Does the central sinus extend through the brain? If not, how far does it extend? Make a longitudinal section through the cerebellum. Find the "tree of life." How many branches do you find? Are the branches simple or branched? Draw.

4. At the base of the brain find the medulla oblongata. Where is the white matter in this? How does it compare with the spinal cord in size and in position of the white matter?

5. Cut away the posterior part of some of the vertebræ in several places and see the spinal nerves. How many roots can you find to each

nerve? See if you can find the sciatic nerve in the hind limb.

6. (Histological.) (L. and H.) **Spinal Cord.**—T. S. With (L.) find in the centre the gray matter. Shape? Is there an opening in the centre for a blood-vessel? With (H.) see if you can distinguish the separate nerves. Also the nerve-cells. Draw.

7. **Brain.**—Find the pia mater, the coat adherent to the brain. How thick is the gray matter? Look for the nerve-cells and nerve-fibres. Draw. How do your nerve-cells compare with those in Fig. 24, Part II.?

8. **Nerve.**—If you have a properly stained nerve, distinguish the myalin and the axis cylinder. Is the fibre of the same diameter throughout its length? Draw. Compare with Fig. 23, Part II.

SPECIAL SENSES.

1. **Tongue.**—Are there any papillæ on the tongue? If so, where are they? Are they all of the same size? If not, where are they the largest? How are they arranged? Draw with the aid of the hand lens.

2. (Histological.) Distinguish, if you can, the different tissues of the tongue. What kind of epithelium covers it? Which way do the papillæ point? Are there any taste buds in any of the papillæ? If so, on what part of the tongue? Shape of the papillæ containing taste buds? Draw a papilla with its adjacent tissue. For structure and place of taste buds, see Fig. 21, Part II.

3. **Eye.**—How many lids are there? Are there eyelashes? Cut away the upper lid to find the muscles that move the eyeball. How many muscles do you find? Take the eye out. Shape? Where is the optic nerve? Draw.

4. With a sharp scalpel or razor cut through the equator of the eye. In the posterior half you may find a thin lining membrane that you can strip away with the forceps, the hyaloid membrane. The front part of this forms the suspensory ligament that supports the lens. Find its attachment round the outer edge of the iris. Besides this, the lens has its own covering, the capsule. Find where the optic nerve enters the eye. Before trying to strip out the hyaloid membrane, see if you can find where

the optic nerve enters the eye. See if you can find the yellow spot. How many coats do you find?

5. Take the lens out. Shape? Lay it on a piece of paper with print on it to see that it magnifies. The iris lays on the lens. Is it attached to it? Color of the iris? Diameter of the cornea? The cavity between the cornea and the iris is filled with the aqueous humor; the posterior part of the eye contains the vitreous humor. What difference do you find between these two fluids? How does the cornea differ from the sclerotica? Draw.

6. (Histological.) (L. and H.) In a section showing the front of the eye find the cornea, and outside this a thin membrane, the conjunctiva, or the mucous membrane of the eye. See if you can find also a thinner membrane on the inside of the cornea, the endothelium layer. Draw. In front of the lens find the iris. (This may not be whole.) See that the back part of this is formed of the back part of the choroidea. Is the iris thickest at the outer or inner part? See that the choroid part of the iris at the outer part is laid in folds. This is

the inner portion of the ciliary process. Draw. See outside this the ciliary muscles between the ciliary process and the sclerotica. See if you can find the two sets of these muscles. These are the muscles of accommodation.

7. With the section of the back part of the eye find the three coats,—sclerotica, choroidea, and retina. If your section shows the optic nerve, which of these coats are absent here? Draw this portion. How many layers or coats can you make out in the retina? See if you can distinguish the nerve-cells and nerve-fibres. Find that the outer layer of the retina is a color or pigment layer but that it is separate from the choroid coat. This is the color layer for the rods and cones. See if you can find the hyaloid membrane. Fig. 25, Part II., may aid in finding the layers of the retina.

8. **Ear.**—How many parts of the external ear do you find? Name them. With the bone-saw cut into the cranium so as to see the middle and internal ears. Find the membrana tympani and the chain of bones. Draw.

DISSECTION OF A FROG.

An alcoholic specimen will be better for this than one that is fresh. If specimens have been kept in alcohol until very hard and stiff, place in a liquid made of equal parts of water, alcohol, and glycerin for half a day or more before dissecting. Dissect alcohol specimens in 50 per cent. alcohol. Skin the specimen carefully, noting the fibres of connective tissue that hold the skin to the parts beneath. Wash away any coagulated lymph which may be found in the depressions between the muscles.

MUSCLES.

1. Without dissecting out any muscles, after skinning, note on the centre of ventral part of abdomen a white line, the linea alba. Note also a muscle each side of this, the rectus abdominis. Just outside this, see the obliquus externus, with the fibres running downward and backward. See also the pectoralis major running from the sternum to the shoulder. Draw these in position.

2. Turn the dorsal side up. Note the depressor mandibuli, posterior to the tympanic

membrane. Notice the latissimus dorsi, posterior to the first. Find the infraspinatus that is partly covered by the latissimus. Find on the posterior part of body the gluteus running from the innominate back on to the limbs. Draw these in position.

3. On the under side of head, extending across from side to side, find the mylohyoid. Draw. Remove this, and find to one side of median line the geniohyoid. On each side, near the angle of jaw, find the air-sac or croaking-bag. Find four small muscles running from this sac to the hyoid bone, the petrohyoids. Draw.

4. On the side of the head find the three muscles of mastication,—the masseter, in front of the union of the upper and lower jaws; the temporalis, running between the eye and the auditory apparatus; the pterygoideus, under the temporalis. Draw.

5. On the foreleg find the brachialis biceps in front of the humerus, and the brachialis triceps back of this bone. Running from the anterior part of sternum to the foreleg, find the sterno-radialis. We do not have this muscle. Draw these three.

6. Find in the hind limb, extending from the innominata to the patella, the rectus femoris, inside this the vastus internus, and running from the pubic portion of the innominata to the knee the sartorius. On the outside is the vastus externus; under the sartorius may be found two muscles, the adductor longus, the front one, near the vastus internus, and the adductor magnus, the posterior one. Pull these with forceps. Result? Use?

7. On the back or under side of the leg, beyond the knee, find the gastrocnemius, with the tendo Achillis attached to the heel. On the anterior side of the limb find the tibialis anticus, one of the muscles opposing the gastrocnemius.

8. (Histological.) Use the paragraph for histology under the dissection of a bird for this.

BONES.

1. Find the humerus. Below or beyond this there is only one bone, the radio-ulna. How does this compare with our arm? See if you can find any indications of two bones. See how many bones of the wrist you can find, carpus, also metacarpus and phalanges.

2. On the hind limb find the femur. How does it differ from the humerus? Draw. Find beyond this the tibio-fibula or leg-bone. How does this compare with the radio-ulna? Draw. Make out all you can of the bones of the foot.

3. In the vertebral column, how many cervical vertebræ are there? The cervical vertebræ extend to the pectoral girdle. By how many condyles does the first or atlas unite with the skull? How many dorsal vertebræ? These extend from the pectoral girdle to the combination of the urostyle and innominate bones. Do these vertebræ have spinous processes and neural arches the same as ours? Draw.

4. Of what bones is the pectoral or front limb girdle composed? Of what is the pelvic or hip girdle composed? Is the urostyle composed of one bone or several? What part of the innominate is represented in the two bones beside this? Draw.

5. What is the shape of the skull as seen from above? Are the eye-sockets large or small as compared with ours? Find back of the sockets the auditory capsule. Find, if you can, the occipital bone, and on the roof of the skull

two bones, the fronto-parietals. Examine the lower jaw or inferior maxillary bones. How do they unite with the cranium? Are there any teeth?

6. Examine, in the occipital bone, the foramen magnum through which the spinal cord passes. Shape? On each side of the foramen magnum find the occipital condyles. In the frog these are borne on each a separate bone, the exoccipital bone. We do not have these. Find them. On the ridge each side find the union of the pro-otic and the exoccipital bone. We do not have this. Find the squamosal bone on each side, extending from the pro-otic down to the posterior end of the upper jaw. Shape? The pro-otic and squamosal bones answer to our temporal bone.

7. What is the relation of the squamosal bone to the tympanic ring? Find under the tympanic ring the columella auris. Trace this in to its inner end, which is closed by a membrane, the fenestra ovalis. Is there any external ear?

8. In front of the frontoparietal bones are sphenethmoid or girdle bones, next the nasal bones with cartilage between them, next the premaxillæ, and last the maxillary bones or

maxillæ. See if you can find all these. On what part of the maxillæ are the teeth? How are all these different from our bones of this part? Draw.

9. Forming the floor of the cranium find the parasphenoid bone. Shape? Find the palatal bones, and in front of these the vomers. The latter bear the vomerine teeth. Draw.

10. (Histological.) For this part take the directions and questions for the histology of a bird.

DIGESTIVE ORGANS.

1. Has the frog teeth in the lower jaw? Find teeth on the vomers, the vomerine teeth. Do you find any other teeth above? What is the shape of the mouth?

2. To what part of the mouth is the tongue attached? Pull it out of the mouth. Is the same surface up when out of the mouth that is when the tongue is in the mouth? Are there any salivary glands? Draw the tongue when in the mouth and when out of the mouth.

3. Is there a crop here as in birds? Find the stomach. What holds the stomach in place? Shape? Draw while in place, including a por-

tion of the alimentary canal above and below. See if you can find the pancreas below the stomach. Handle carefully, and find the ducts by which the pancreatic juice and bile enter the duodenum. How far apart are they?

4. Are the intestines long or short? How many times as long as the body? Is there a distinction in size between the large and small intestines? The coiled portion is the small intestine. What holds the intestines in place?

5. Where is the spleen? Cut open the stomach. What is in it? From what you see what is the food of the frog? Is the inner coat of the stomach smooth or in folds?

6. (Histological.) **Tongue.**—What difference is there in the tissue of the upper and lower surface? Are there any papillæ? If so, where? Any taste pits? See if you can find with the high power mucous glands. Draw. For structure of taste buds or pits, see Fig. 21, Part II.

7. **Stomach.**—How many layers of muscles do you find in the muscular coat? Find the peritoneum on the outside. Is there any difference between the muscular and submucous layers, the latter just below the glands? Shape

of the glands? Draw a section showing these. What difference in the glands in the two ends of the stomach?

8. **Intestines.**—Find the peritoneum, the longitudinal and transverse muscular fibres. The villi form the inner layer of gland-like organs, and just outside these the glands of Lieberkühn. Find both of these. With the high power find the cells of these glands. How do these compare with Fig. 20, Part II.? Draw.

RESPIRATION.

1. Where are the nostrils? Into what do they open? Find the trachea. Is there a larynx? If so, shape? Are there cartilaginous rings in the trachea? If so, are they entire or open on one side? Length of the trachea?

2. How far back do the lungs extend? Shape? How long are the bronchi? If the lung is collapsed, insert a blow-pipe into the bronchus and inflate it. Difference in size between the inflated and collapsed lung? Look for the pulmonary artery running the length of the lung while it is inflated. Are the cells large or small?

3. Open the glottis and look for the vocal cords, two narrow bands. If the frog is a male (which may be known by the fourth digit on the anterior foot being much enlarged), find each side in back part of the mouth the vocal sacs or croaking-bags. Size? Shape? Draw.

4. (Histological.) In the trachea find the different coats of which it is composed. Name them, and draw. See if you can find any ciliated epithelium lining the inner coat. Use (H.) for this. Note the size of the lung-cells. Find the outer coat or pleura. Draw.

CIRCULATION.

1. If a live specimen can be had, make a narrow opening in a piece of card-board or other thin substance, tie a thread to the two longer toes of one hind leg, gently stretch the included membrane over the opening in the card-board, fastening the threads to the sides of the board with the frog on the board, ventral side down. Securely fasten the frog to the board, but do not fasten the leg too tightly whose membrane you are to examine, or it will stop the circulation of the blood. Place the

board, with the frog on it, under the clamps of the microscope stage so that the web comes under the objective. If the class is large, so that much time will be consumed in the examination, put a drop of water on the web, and place over this a narrow piece of cover-glass. Focus so as to see the capillary circulation. Does it flow in waves or as a continuous flow? Are the vessels of the same size or are some larger than others? If the latter, can you distinguish by the flow which of the larger are arteries and which veins? Draw.* (If the frog becomes dry, a moist cloth may be placed on its back to keep it moist.)

2. If the frog is alive, place it in a jar with a piece of cotton wet with chloroform. As soon as it is dead, place it in a dissecting-pan on its back. Carefully open the skin the whole length of the body, then along the limbs, and bring the flaps of skin back and pin them. Carefully

---

* The tail of a large tadpole may be used to show the circulation. Wrap the tadpole in a wet cloth, leaving the tail out, and lay on a plate of glass. Place this under the stage-clamps so that the tail comes under the objective. Keep moist, and replace, and focus if the tadpole moves.

cut through the sternum and abdominal wall and turn these back. It is probable that the heart will still be beating. What is its position in regard to the sternum? What in regard to the lungs? Note the pericardium. Does it fit the heart closely or not? Does it contain anything but the heart? Color?

3. Slit the pericardium and remove it. Shape of heart? Color? Draw, including the vessels. How many auricles? How many ventricles? On the ventral surface of the heart find a single vessel, the truncus arteriosus or aorta, which soon divides into two aortic arches. Below find a large vessel, the inferior vena cava, extending from the liver to a thin-walled sac, the sinus venosus. Properly, this should not be called the inferior vena cava till after it has united with the veins from below, the renal and genital veins. Into the same sinus venosus empty also the two superior venæ cavæ. Find, and designate all these on your drawing.

4. Find the hepatic vein and trace to its union with the veins from below. Find the portal vein. Of how many lobes is the liver composed? Where is the gall-bladder?

5. (Histological.) With a section of the heart find the adherent portion of the pericardium. Also the endocardium. Is there any partition in the ventricle? Are the valves muscular or membranous? Is there any sarcolemma?

NERVOUS SYSTEM.

1. To examine the brain and spinal cord, a specimen should be taken that has lain in alcohol a few days. Clean away all the skin and muscles from the back. Remove carefully the roof on the skull without touching the brain, and cut the neural arches on each side of the spinal column and remove them. To show the sympathetic system, take the ventral portion of the frog and remove the viscera from one side or push it to one side.

2. What is the shape of the brain? Size? Are the two sides alike? Is it convoluted? Find the cerebral hemispheres. Anterior to these find the two olfactory lobes. These give off the olfactory nerves. Shape of both these parts? Back of the hemispheres find a diamond-shaped area, the thalamencephalon. Inside this find a cavity, the third ventricle. The

sides of the thalamencephalon form the optic thalami. Next behind this area are the optic lobes. Size? Shape? Back of these is the cerebellum, a transverse band. The last after this is the medulla. Find all these if you can, and designate them on your drawing.

3. Size of the spinal cord as compared with the brain? Find the enlargement where the nerves of the front limbs are given off. The second and third spinal nerves form the nerves for these limbs, or the brachial plexus. The seventh, eighth, and ninth form the sciatic nerve, or the plexus for the hind limbs. Is there a dorsal fissure in the spinal cord? Draw.

4. Find the sympathetic system as two cords on the sides of the spinal column in the visceral cavity. See if you can find the connections of this with the viscera by nerve-fibres.

5. (Histological.) The parts given for the histology of the nervous system of a bird may be used here.

SPECIAL SENSES.

As the organs of special senses are so small in the frog, it will be better not to attempt a general study of these in this animal. The

following may be studied histologically with profit, however. The eye may be prepared whole and sectioned perpendicular to the retina. The cells of this coat are large, and form a good study with the high power. For the general study of the eye, however, the cat's eye is the best.

The end organs of the olfactory nerve of the frog show distinct bristles beyond the supporting epithelium cells. A transverse section through the nostrils should show these in good shape. In mammals these end organs scarcely protrude beyond the supporting cells.

PART II.

HISTOLOGY.

SOME PREPARATIONS THAT ARE NEEDED.

Alcohol.—This, as a hardening agent, is used in the following strengths: 35 per cent., 50 per cent., 70 per cent., and 95 per cent. In some special cases 100 per cent. alcohol is used for hardening. The tissues should remain in the different strengths of alcohol twenty-four hours or more.

Alcohol as a wash is used in the following strengths: 35 per cent., 50 per cent., 70 per cent., 95 per cent., and sometimes 100 per cent. In treating sections in celloidin the 100 per cent. alcohol may be omitted, or if used it should be poured on and off as quickly as possible or it will dissolve the celloidin. 100 per cent. alcohol is absolute alcohol.

Celloidin.—For the purpose of embedding, celloidin is used in two or three strengths. If properly prepared, two are all that are needed.

Thick Celloidin.—Dissolve till syrupy celloidin in equal parts of ether and alcohol. 95 per

cent. alcohol will answer, but 100 per cent. is much better.

Thin Celloidin.—This is one volume of thick celloidin in two volumes of ether-alcohol.

Clearing Oil.—Usually only one form of clearing oil is needed, and 90 c.c. of that is made as follows:

Carbolic acid, crystallized. 30 c.c.;
Cedar-wood oil, 30 c.c.;
Oil of bergamot, 30 c.c.

Melt the carbolic acid and mix. After the clearing oil has been used once it may be filtered off and used again. Some of the aniline stains require a special clearing oil, but these will be spoken of when required. If the clearing oil dissolves the stain out of sections, do not use this again for any other stain.

Acid Alcohol.—The usual preparation of this is as follows:

Alcohol, 70 per cent., 100 c.c.;
Hydrochloric acid, 1 c.c. or 6 drops.

In some special cases it is made from 95 per cent. alcohol, and in others it is made from 100 per cent. alcohol.

Fixative.—If celloidin is used for embedding, no fixative is necessary; but if paraffin is used, it is necessary to fix the sections on the slide and afterwards dissolve the paraffin from the sections. A good fixative is made of equal parts of the white of an egg and glycerin. Filter the white of the egg before mixing. Spread a thin film of the fixative over the slide, and carefully place the section where it is wanted. Warm gently, but not enough to melt the paraffin, and then dissolve the paraffin in turpentine or xylol.

Müller's Hardening Fluid.—This contains the following ingredients:

Potassium bichromate, 25 grammes;
Sodium sulphate, 10 grammes;
Water, 1000 c.c.

Pulverize the solids before placing them in the water. Place a piece of camphor gum in the solution to prevent moulding.

Picrosulphuric Acid.—This decalcifying fluid is made as follows:

Saturated watery solution of picric acid, 100 c.c.;
Sulphuric acid, 2 c.c.

Mix the ingredients, and after twenty-four hours filter and add 300 c.c. of water to the

filtrate. This may be used for a hardening fluid as well as for decalcifying. For this purpose the tissues should remain in the fluid only a short time, never to exceed six hours. Complete the hardening in alcohol.

Chromic and Nitric Acid Fluid.—This is an excellent fluid for decalcifying small bones, as the jaw and teeth of a cat or a rat, or the vertebræ of the same. The bones should remain in the fluid from two to four weeks, and the fluid should be changed several times in that time. Mix:

Chromic acid, 1 gramme;
Water, 200 c.c.;
Nitric acid, 2 c.c.

Formic Glycerin.—Mix the following:

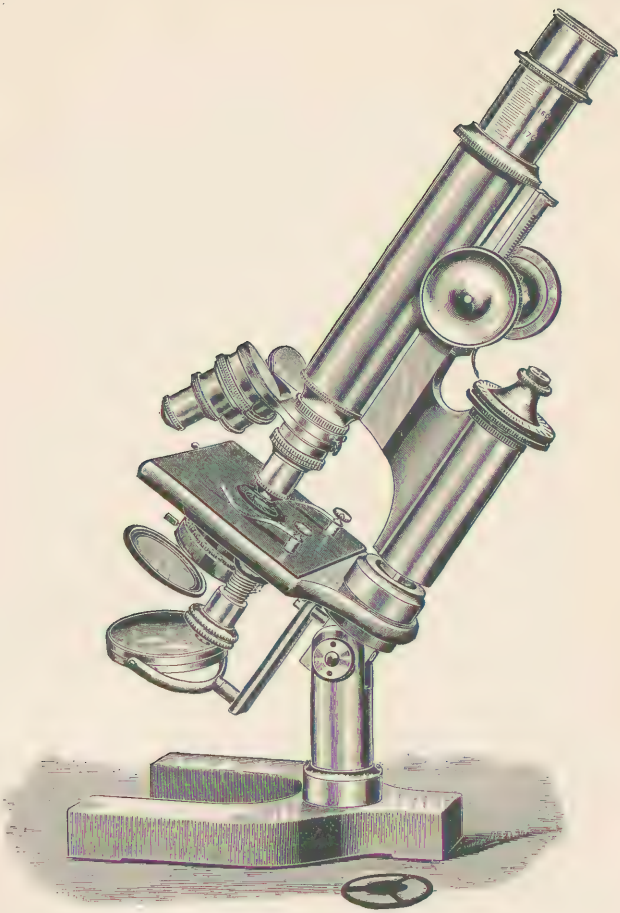
Dilute glycerin, 15 c.c.;
Formic acid, 1 drop.

Dilute Glycerin.—This is made of equal parts of distilled or filtered water and glycerin.

THE MICROSCOPE.

Fig. 1 represents what the Bausch & Lomb Optical Company, of Rochester, New York, call their "BB8" microscope, one-half the actual

FIG. 1.



The microscope. ×⅓.

size. This instrument as represented in the figure has three objectives, a two-thirds, one-sixth, and one-twelfth oil immersion, and two eye-pieces, a two-inch and a one-inch. Good laboratory work can be done by leaving off the one-twelfth oil immersion objective. Besides these the figure represents under the stage an Abbe condenser and iris diaphragm.

As the figure is not lettered, we will explain it without lettering. The lower part that forms a support for the instrument is called the base; the upright piece that is attached to this is called the standard, and the two make the stand. This forms a support for the essential parts of the instrument. The flat part at right angle to the standard and above the joint is called the stage. On the upper side of this are the clamps that are used to hold the slide to the stage. To the left of the standard and attached to it is the tube. The objective is usually inserted into the lower end of the tube. This figure shows three objectives fastened to the tube by a nose-piece. By this device the objectives may be used successively by turning the nose-piece without unscrewing the objective

from the tube and inserting another. The terms two-thirds, one-sixth, and one-twelfth, when applied to objectives, mean that the focal distance of these when used singly is respectively these parts of an inch. When the magnification is increased by the use of the eye-piece the focal distance is shortened. The first two of these are used without any medium but air between the objective and the cover-glass covering the object examined. In using the third a drop of cedar-wood oil is placed on the end of the objective, and this brought down so as to touch the cover-glass, making a liquid medium between the objective and the cover-glass.

The upper end of the tubes has inserted into it the eye-piece or ocular. Besides this, it has what is known as the draw-tube, that may be raised or lowered. This is shown partly raised, the scale and figures showing the part that is concealed when the tube is lowered. To raise this to its full length gives what is called full-tube length, or two hundred and sixteen millimetres, when we have the greatest magnification the instrument is capable of with a given combination of eye-piece and objective. When the

draw-tube is down, the tube length is one hundred and sixty millimetres, or what is called short-tube length. The Bausch & Lomb catalogues and the back of their microscope-cases give a table showing the amount of magnification for full-tube length and also for short-tube length for each combination of eye-piece and objective.

The milled heads at the right of the tube are for turning the coarse focussing, and are called, with the shaft connecting, the rack and pinion. By this device the tube is rapidly raised or lowered. The milled head at the end of the standard is for fine focussing, and is called the micrometer screw.

Below the stage is first the mirror for reflecting light up through the object. In this instrument this is a double mirror, one side plane and the other concave. This is attached to the standard by a swinging bar. Above the mirror is the combination of the Abbe condenser and iris diaphragm, the diaphragm being the lower part of the combination. This is focussed in place or turned to one side by the double-milled head that shows near the mirror. A round

frame attached to this is for holding a round blue glass that is to be used when using the microscope at night by lamp-light. When not in need of this, the blue glass should be taken out and the frame turned under the diaphragm. At times it may be necessary to examine specimens by direct light. At such times the circle with dark centre shown at the bottom of the figure may be placed in the frame instead of the blue glass.

USING THE MICROSCOPE.

1. Before taking the microscope from the cabinet, place the piece of chamois-skin on the table to place the microscope on to avoid scratching the table. Set the instrument so that it is square with the operator, with the tube from him, and adjust the mirror so that clear light from a window is reflected through the tube.

2. Before putting anything on the stage for examination, see that the low-power objective is under the tube and high enough, so that the objective will not touch the object on the slide. When through work, leave the instrument with the low-power objective under the tube. This

rule implies the use of a nose-piece on the microscope.

3. Temporarily, a substance may be examined with the low power without a cover-glass over the object, but as a general principle always use a cover-glass. In using stains or reagents, do not wet the upper side of the cover-glass with the fluid, as this would be liable to soil the objective.

4. A drop of fluid placed on the slide by the edge of the cover-glass may be drawn under the cover-glass by touching the edge of a piece of filter-paper to the fluid on the opposite side of the cover-glass. Surplus stains or reagents may be washed out in this way,—by a drop of water added to one edge of the cover-glass and drawing it through with the filter-paper.

5. In handling objectives and eye-pieces, never touch the glass part with the fingers. In handling slides and cover-glasses, do not touch the flat surfaces with the fingers, but hold them by their edges.

6. Examine objects first with the low power, and always use the low power as a finder for small objects.

7. Keep both eyes open while looking into the microscope. This can be done after a little practice, and avoids fatigue to the unused eye. Get into the habit of using either eye, as it is better for the eyes.

8. Regulate the amount of light needed by the use of the diaphragm. The light and focussing should be such that you get a clear view of the object examined. Do not be satisfied with anything short of this.

9. Do not allow fluids to get on the stage of the microscope. In mounting objects for the microscope, whether for temporary use or to preserve, use only as much of the mounting media as will just fill the space under the cover-glass, not a surplus to run over the slide.

10. In focussing, use the rack and pinion for coarse focussing and the micrometer screw for fine focussing. At first adjust the focus of the condenser, and afterwards leave it without further altering it.

11. In drawing objects from the microscope, note the magnification. If drawn the same size as seen, this will be the combined magnification of the eye-piece and objective used. If drawn

larger than seen, multiply this by the number of times larger. Do not make your drawings small.

12. In changing from the lower to the high power, if the microscope has a nose-piece, it is better to turn the tube up a very little before turning the high-power objective round.

HARDENING.

Most tissues should be hardened before attempting to cut them into sections, and a few, as bones and teeth, need to be decalcified as well as hardened. For most organs, alcohol in its various strengths is the best and the only hardening fluid needed. Alcohol may be used for all the organs and produce very good results. Where any other fluid will give better results it will be given in the different schemes.

Before hardening, tissues should be cut into small pieces, from a quarter to half an inch in diameter is large enough. Each specimen or part of an organ should have a small tag, not more than a quarter of an inch square, attached to it by a thread. On this tag should be a number that corresponds to a number on a list

that is kept of the different tissues that are put into the hardening fluid. This tag should remain on the specimen till it is ready to cut into sections, and hence should be so placed as not to interfere with the sections that are to be made. Parts of the stomach and skin should be spread out flat, and if necessary pinned to sheet cork with thorns.

Alcohol.—Fresh tissues should be placed first in 35 per cent. alcohol for twenty-four hours. After this place them successively in 50 per cent., 70 per cent., and 95 per cent., twenty-four hours in each. They will be benefited by remaining in the last longer than the twenty-four hours.

Chromic Acid.—To 1 gramme of this add 200 c.c. of distilled or filtered water. This is specially useful in hardening such tissues as the nerves, eye, etc. After treating tissues with chromic acid the hardening should be completed in alcohol, beginning with 50 per cent.

Müller's Fluid.—The composition of this is given in the chapter on preparations needed. Where a long time can be given to the hardening, this is an excellent preparation. It hardens tis-

sues completely and with scarcely any shrivelling. Blood-corpuscles retain their shape and, if not stained, their natural color. For its use, see Scheme 11.

Corrosive Sublimate.—Many tissues will be benefited by placing in this first and completing the hardening in alcohol. A saturated solution can be kept in stock by placing more than will dissolve of the crystals in a bottle and filling the bottle with water. It hardens rapidly. For its use, see Scheme 5.

Chromic acid and all chromine compounds used in hardening or decalcifying should be kept in the dark while in use, and also the specimens partly hardened in these compounds should be kept from the light after leaving these for the alcohol. Picrocarmin will be the best stain for tissues hardened in chromic acid or its compounds.

DECALCIFYING.

Before they can be cut with the microtome bones must be freed from their mineral matters. While this is being done, the fluid should be such that the tissues will not be destroyed nor changed more than in ordinary hardening. Only

small parts of the tissue to be decalcified should be placed in the fluid, and the quantity of the fluid should be large in proportion to the specimens. After decalcifying, all of the decalcifying fluid should be washed out of the specimens before they are placed in alcohol. The hardening should be completed in alcohol, beginning with 50 per cent. or 70 per cent.

The following are some of the more commonly used decalcifying fluids:

CHROMIC ACID.

CHROMIC AND NITRIC ACID FLUID.

PICRIC ACID, saturated solution.

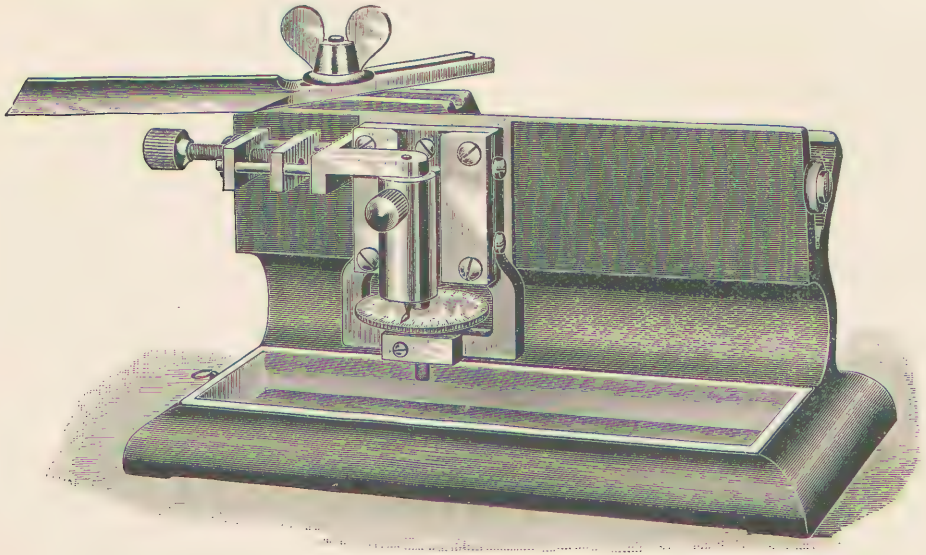
CHROMIC ACID AND HYDROCHLORIC ACID FLUID.

The preparation of these will be found in the chapter on "Some Preparations that are Needed." The time that a bone should be left in the decalcifying fluid is given in the schemes where they are to be used. Generally the time given there is sufficient, but the specimen can be tested by seeing if a pin will enter it readily.

THE MICROTOME AND ITS USE.

Fig. 2 represents the "student microtome," an inexpensive and yet a very serviceable instru-

FIG. 2.



The student microtome. ×⅓.

ment. At the left is shown the knife in position for cutting sections where the material to be cut is embedded in celloidin. Where the material for embedding is paraffin, the knife is set at right angles to the plane of motion. Below the knife is the clamp for holding the embedded specimen to be cut, with a milled head for turning the screw that moves the clamp. To the right is another milled head that turns a screw that sets the clamp after it is turned round where it is wanted. The dial below is a gauge by which the thickness of the sections may be determined. A turn of ten degrees to the right on the gauge is necessary to cut a section of $\frac{1}{1000}$ of an inch in thickness, etc.

In using the microtome, first see that the screw attached to the gauge is turned down and this carries the clamp with it. Fasten the cork or block holding the specimen into the clamp at such a height that the top of the embedded specimen will just come to the knife. Have bearings of the knife-carrier oiled so the motion of the knife is easy. With the knife to the left, and set obliquely, turn the gauge to the right as many degrees as desired, and draw the knife to

the right. Do not move the gauge till the knife is carried back to the left again. Keep the knife and specimen wet with 70 per cent. alcohol while cutting sections embedded in celloidin. When the cutting is completed, the knife should be thoroughly cleaned and returned to its case. Always strop the knife before using it. In honing the knife, always hone it on the concave side. After the honing is completed, draw the knife across the hone once on the flat side.

EMBEDDING.

Tissues in their fresh condition are too soft and flexible to be cut into sections thin enough for study with the microscope. Even after they have passed through a regular process of hardening, they are still too flexible. To overcome this, tissues are sometimes frozen and cut while in this state, but the usual method is to embed the tissue in either paraffin or celloidin and cut it while in this condition. It is thought best by the writer to give only the celloidin process of embedding here, as that is simpler and requires less apparatus than the other.

After the specimens are hardened, place them

for twenty-four hours in thin celloidin. By this time the celloidin will penetrate all parts of the tissue, though from its softness it may not seem to do so. Next place the specimen in thick celloidin for twenty-four hours. This replaces the thin celloidin in at least the larger open spaces. At the end of the second twenty-four hours the tisssue is ready for embedding.

To embed, have a cork or a short block,—an inch long is long enough,—whose diameter is a little more than that of the specimen. Wet the end of the block or cork with the thick celloidin and allow it to stand perhaps half a minute. Now place the specimen on this in such a way that when cutting the sections they will be as desired. As, for example, if a cross-section of the trachea of a cat is desired, the end of the trachea should rest on the cork. After placing the specimen on the block in the celloidin, allow it to stand about a minute and then pour thick celloidin over this. This will make a coat of the celloidin over the specimen and down onto the top of the cork. After this has stood long enough to set a little, pour more celloidin over it again, and repeat the operation till there is

enough on the specimen and the cork to hold the specimen in place while cutting, and to hold the sections together after they are cut. Now let it stand till the celloidin is somewhat thickened, which will take about five minutes, and then place it in 70 per cent. alcohol, where it should remain twenty-four hours. This hardens the celloidin.

Before beginning to embed, it is well to add a few drops of ether to the celloidin and shake the bottle. This will compensate for what may have evaporated through the cork. Also, keep the celloidin bottle corked when not using it.

STAINING.

Without staining, tissues of different kinds look very much alike, except the variations of the different parts. The hardening materials take the coloring out of tissues, so that after they have been passed through all the chemicals necessary for hardening, embedding, and cutting sections are too pale to be seen well. Staining does not give them their natural color, but it gives them a color so that the parts of the section may be readily seen. The objects of stain-

ing, then, are to render the parts of a section more easily seen and to show the different kinds of tissues of which a section is composed. The latter is best done by double- or triple-staining.

Stains may be divided into nuclear and perinuclear. If a nuclear stain is used alone, it generally stains the section of one color if it be a section of an animal. If, after the nuclear stain has been used, a perinuclear stain be used, the first stain is replaced in the section in all parts but the nuclei, and these alone retain the first color. This is called double-staining. Sometimes the two stains are applied separately and at others they are both applied at the same time. As staining reagents can be obtained now ready prepared, the writer will say nothing here about the preparation of the dyes.

SIMPLE STAINING.

Boraxcarmin.—This is the best simple stain we have for the most of tissues, and it is one of the best perinuclear stains. It may be used from 70 per cent. alcohol, after which remove the surplus stain with acid alcohol. See Scheme 1 for this.

Hæmatoxylin.—This may be used for a simple stain or for a nuclear dye in multiple staining. Pass the sections from the 70 per cent. alcohol through 50 per cent. alcohol into water, and as you pour off the water leave a little in the stender dish to dilute the stain. Color the sections till they are blue-gray. Wash with water and pass through the strengths of alcohol to the clearing oil. Do not overstain.

Safranine.—This is an excellent simple and perinuclear stain. Pass the sections from the 70 per cent. alcohol into water and stain with dilute solution. After staining, the sections may be passed back to 70 per cent. alcohol and washed with acid alcohol, or the acid alcohol may be omitted. The washing should be rapid or too much of the stain will wash out.

Eosin.—This color may be had in powder or liquid form, the powder being in the end the cheapest. It is easily soluble in water, alcohol, or clearing oil. For staining blood-corpuscles dissolve enough in a small quantity of water to obtain the desired depth of color, and use a pipette to put the dye on the cover-glass. If sections are stained in eosin, they should pass through the

washes rapidly, or too much of the dye will wash out. Eosin is used more for a perinuclear stain than for simple staining, except in case of epithelium-cells and non-nucleated blood-corpuscles.

Aniline Dyes.—There are many of these, only a few of which may be mentioned here. Among the best are gentian violet, aniline blue, methylene blue, iodine green, aniline green, methyl green, gold-orange, fuchsin, acid fuchsin, and safranine and eosin, already spoken of. In general, these do not stain tissues in the proper sense of that word, but rather the tissues imbibe the dyes, for which reason they may be rather easily washed out. For this reason, after staining in aniline dyes, sections should be rapidly passed through the washes to the clearing oil. This will wash out some of the dye, hence the clearing oil from one of these stains should not be used for any other color, but if filtered out should be kept for the same color. The aniline dyes are, as a rule, perinuclear stains.

Silver Nitrate.—Nothing need be said of this here only that it is used to show the boundaries of the cells of endothelium. Its use is explained in Scheme 7.

DOUBLE-STAINING.

The best method of differentiating tissues is by double- or triple-staining. This is done sometimes by mixing the dyes in one fluid and applying this to the sections, or by staining with the dyes successively. In either case the different parts of the section take the different stains, and this fact shows the composition of the section. Multiple staining requires careful manipulation, and should not be undertaken till the student is thoroughly familiar with simple staining. The following are some of the more easily managed double stains:

Picrocarmin.—This stands at the head of double stains, and should be used where chromic acid or its compounds have been used in hardening or decalcifying. Stain from 70 per cent. alcohol, leaving a very little of the alcohol in the stender dish to dilute the stain, but adding a very small quantity of picric acid before putting in the dye. After staining add a little picric acid to each wash, as per Scheme 9.

Hæmatoxylin and Boraxcarmin.—Stain first with the hæmotoxylin till the sections are blue-gray, wash with water, pass to 70 per cent.

alcohol, and stain with the boraxcarmin. This will give the nuclei blue and the perinuclear parts red.

Hæmatoxylin and Gold-Orange.—Stain first with the hæmatoxylin, wash with water, and pass back to 70 per cent. alcohol the same as with the preceding. Add the gold-orange after pouring off the alcohol and allow to stand five minutes. Pour off and wash rapidly with 70 per cent. and 95 per cent. alcohols and put on the clearing oil. This combination is specially useful in showing the nuclei and bands of voluntary muscles, and for showing the nuclei in such tissues as the kidney or spleen.

Hæmatoxylin and Safranine.—Treat the same as with the preceding.

Hæmatoxylin and Fuchsin.—Treat this the same as hæmatoxylin and gold-orange.

Hæmatoxylin and Eosin.—Treat this the same as hæmatoxylin and gold-orange.

Eosin-Methylene Blue.—This is a fluid ready prepared. Stain from 70 per cent. alcohol, and after staining wash rapidly with 70 per cent. and 95 per cent. alcohols and add the clearing oil. This combination is good for sections of

the ear of a cat or rat, as the cartilage will be stained blue and the skin pink. It is also useful for glandular tissues, such as the stomach, tongue, etc. This stain easily washes out, hence to get good results the sections should be mounted as soon as possible after putting into the clearing oil.

Ehrlich-Biondi-Heidenhain. — This multiple stain is recommended in staining sections of the spinal cord in Scheme 14. Unless properly done any other stain will be better than this. It may be operated as recommended in that scheme, or a strong solution of the stain may be on the sections for a few hours without heating. In that case watch the sections to see when sufficiently stained. If the mounting be done at once the ordinary clearing oil may be used. It makes a good stain for the spleen and also for the gizzard of a bird, as it shows in the first case the trabeculæ of the capsule pink while the other parts are differently colored. In the gizzard the muscles will show the transverse marks with the low power, so characteristic of some of the involuntary muscles in wavy red lines, while the muscle-fibres will be green. In

sections of the liver it stains the capsule and the cell boundaries red, while the other parts are greenish. Even when the stain is washed out so as to be red the boundaries of the cells may be seen. The stain is to be had in a powder. Dissolve this in water and filter. Stain in dilute solution for twenty-four hours or stain by heating.

Indigocarmin.—This is to be had in the form of a waxy mass. Dissolve a small amount of this in water, and add about one-fourth as much boraxcarmin solution. For the rest of this follow Scheme 11.

MOUNTING SECTIONS.

After sections have been stained, dehydrated, and passed into the clearing oil, they should remain in this long enough to replace the alcohol with the clearing oil and render them transparent. The slide and cover-glass should be thoroughly cleaned. In placing the sections on the slide some may be taken with the forceps, but if they fold up in doing this, take them out of the clearing oil with the section-lifter. Place the section in the centre of the slide, remove

the excess of clearing oil with a piece of fine blotting-paper or filter-paper, put a drop of balsam on the section, and over this place the cover-glass. If the clearing oil has run out over the slide, this should be removed by wiping with the clean edge of the filter-paper. It may be necessary to press the cover-glass gently after putting it on the balsam to force the small air-bubbles out from under the section. Just enough balsam should be used to fill the space under the cover-glass, as too much will make an unsightly ring round the glass. A little experience will show any one how much will be needed. Endeavor to make clean, nice-looking mounts.

Canada balsam is the best medium for making permanent histological mounts. The best way to use this is to have it dissolved in xylol, or what is known as xylol balsam. This can be had in tubes ready prepared, and the most convenient way to use it is to keep it in the tubes with the cap on when not using it. If it is not desirable to make a permanent mount, a section can be examined in water, glycerin, alcohol, or normal salt solution, according to the conditions.

After the section is mounted it should be labelled. A convenient way to do this is to place a square label across one end of the slide, the right-hand end being preferred. The label should contain the name of the organ of which it is a part, the animal from which taken, and the stain used. If the section is double-stained, the order in which the dyes are used is suggested. As, for example, "Lung, Rabbit, Hæmatoxylin, Gold-Orange," with each word on a line, with a line between the first and second and second and third is a plan used by the writer.

INJECTING SPECIMENS.

Very little of the circulation can be studied, except the larger veins and arteries and the heart, unless some colored matter is introduced into the circulatory system. The syringe for this purpose the writer has not figured, for the reason that a good figure is to be seen in catalogues of microscopic materials. Gelatin colored with carmin or Berlin blue can be obtained for this purpose already prepared. Just enough of this should be dissolved in water that when cold it will be jelly, and when as warm as

can be borne by the hand will be fluid. It should be in this hot condition while in use.

To inject an animal, kill it with chloroform, and as soon as dead begin the injection at once before the blood coagulates in the circulation. Open the thorax by cutting the ends of the ribs on one side; cut into the right auricle to let the blood out as it is forced through the system. Next cut off the end of the heart so as to reach the left ventricle, insert the end of the canula into this and pass it into the aorta. With a threaded needle pass a thread round the aorta and tie this tightly to the canula, after which pass the thread through the hook or ring on one side of the canula and tie it again. This will keep the aorta from slipping off from the canula while the injection is going on. The animal should now be placed in water about blood temperature and kept there while it is injected. Fill and empty the cylinder of the syringe several times with hot water to get it of the temperature of the injection medium, after which fill it with the gelatin. Now insert the nozzle of the syringe into the canula and begin the injection. Do this with a gentle but steady pressure, keeping

the syringe and the animal in the hot water. To make a good injection will take from fifteen to twenty minutes.

If the syringe will not hold enough once full to complete the injection, place the stop-cock on the nozzle of the syringe before inserting it into the canula, as when the cylinder is empty this may be turned, the syringe taken off and filled and placed back, and the injection completed. After the injection is completed, place the animal in cold water, where it should remain for several hours, till the animal is cold, which will set the gelatin. Then it may be cut up and the pieces put into alcohol for hardening, if injected for this purpose, or it is ready for dissection.

SCHEMES FOR HISTOLOGY.

SCHEME ONE.

To Prepare Tissues for Mounting.—Tissues of animals should be as fresh as possible, and cut into small pieces; from a quarter to half an inch is large enough. The scheme for all ordinary tissues is as follows:

Alcohol, 35 per cent., 24 hours.

Alcohol, 50 per cent., 24 hours.

Alcohol, 70 per cent., 24 hours.

Alcohol, 95 per cent., 24 to 48 hours or more.

Thin celloidin, 24 hours.

Thick celloidin, 24 hours.

Embed in thick celloidin, put in 70 per cent. alcohol, 24 hours.

Cut, putting sections in 70 per cent. alcohol.

Pour off alcohol and stain for from 1 to 5 minutes.

Acid alcohol, 1 to 2 minutes, shake gently.

Alcohol, 70 per cent., shake gently.

Alcohol, 95 per cent., shake gently.

Clearing oil, shake gently.

Mount in xylol balsam.

While cutting keep the knife and the specimen wet with 70 per cent. alcohol. Boraxcarmin is the best stain for most of the tissues. Where a particular stain is more suitable for a part, it will be spoken of in treating of that part, and to which the student is referred. The student is referred to the chapter on staining for the use of particular dyes. If the hardening liquid becomes turbid or bloody it should be changed, the tissues being put into fresh liquid. Before beginning work on this page the student

should study carefully the chapters on hardening, embedding, and staining, as well as the one on the microtome.

SCHEME TWO.

To Stain Tissues in Bulk.—Harden as directed in Scheme 1. Then, after hardening, place them in—

Boraxcarmin, diluted with 70 per cent. alcohol, 24 to 48 hours.

Acid alcohol, 1 to 5 hours, according to size.

Alcohol, 70 per cent., 5 minutes to ½ hour.

Alcohol, 95 per cent., 5 minutes to ½ hour.

Thin celloidin, 24 hours.

Thick celloidin, 24 hours.

Embed and put in 70 per cent. alcohol for 24 hours.

Cut, putting sections into 70 per cent. alcohol.

Alcohol, 95 per cent.

Clearing oil.

Mount in xylol balsam.

This is a good method of treating small pieces of many tissues, especially such as the small intestine of a cat or bird, where the stain can get to the interior of the specimen as well as on the out-

side. If the specimen is large it will not readily stain through by this process, and for such it is better to use Scheme 1.

SCHEME THREE.

Cover-Glass Preparation of Nucleated Blood.—The blood of a frog, bird, snake, or of any animal with nucleated blood may be used for this. Put a drop of the blood on a clean cover-glass and put another cover-glass over this. After gently pressing the glasses together, separate them and put each in a cover-glass-holder with the blood side up. A very thin film of blood may be put on the cover-glass without putting another over it, but it should be very thin. The scheme in detail is as follows:

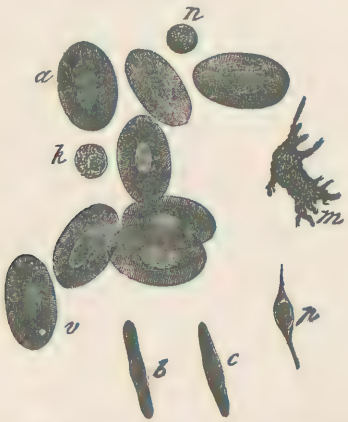
Blood on cover-glass.
Dry.
Methyl green on blood, 5 minutes.
Dip in water once.
Touch edge of cover-glass to filter-paper.
Dry.
Eosin, 5 minutes.
Dip in water once or twice.
Touch the edge of cover-glass to filter-paper.

Dry.

Mount in xylol balsam.

To mount, put a small drop of the balsam on a slide and put the cover-glass on this with the blood side down. Specimens prepared in this way should show the nuclei pale green and the perinuclear part of the corpuscle red. If washed too much the green will be washed out of the corpuscles.

FIG. 3.



Blood of a frog: *a*, red corpuscle seen flat; *b*, seen in profile; *c*, seen obliquely; some of the red corpuscles show vacuoles, as *v*; *n*, white corpuscle at rest; *m*, one with amœboid pseudopods; *p*, a cell probably from the blood-vessel.

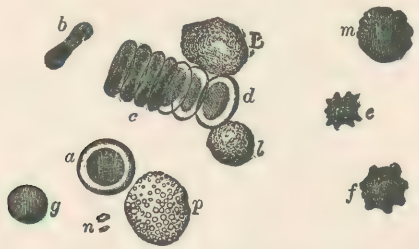
Fig. 3 shows the blood of the frog as it would be if prepared in this way. Besides the colored

corpuscles, there will be white corpuscles present that are of a different shape and smaller. In the figure, *n* and *k* show two of these at rest, while *m* shows one with the pseudopods extended; *b* and *c* show the corpuscles as seen edgewise.

SCHEME FOUR.

Cover-Glass Preparation of Non-nucleated Blood.—The blood of man or of any mammal will answer for this. Prepare the cover-glasses the same as in Scheme 3. The scheme in detail is as follows:

FIG. 4.



Human blood: *a*, red corpuscle seen flat; *b*, seen in profile; *c*, a rouleau; *d*, three-quarter face; *e*, *f*, crenulated corpuscles; *g*, spherical; *m*, slightly crenulated; *L*, large white corpuscle; *l*, small white corpuscle; *p*, granular leucocyte; *n*, granules. × 1000.

Blood on cover-glass.

Dry.

Eosin, 5 minutes.

Wash gently in water.

Touch edge of cover-glass to filter-paper.

Dry.

Mount in xylol balsam.

Mount the same as directed in Scheme 3. The characters of human blood are seen in Fig. 4. Besides being much smaller than the corpuscles of the frog, they are round and not nucleated.

SCHEME FIVE.

To Prepare Sections by the Use of Corrosive Sublimate.—Place small pieces of tissue in—

Corrosive sublimate, 1 to 5 hours.

Alcohol, 70 per cent., 24 hours.

Alcohol, 95 per cent., 24 hours, or longer.

Thin celloidin, 24 hours.

Thick celloidin, 24 hours.

Embed, putting in 70 per cent. alcohol, 24 hours.

Cut, putting sections into 70 per cent. alcohol.

Boraxcarmin, 1 to 3 minutes.

Acid alcohol, 1 to 3 minutes.

Alcohol, 70 per cent., shake gently.

Alcohol, 95 per cent., shake gently.

Clearing oil, shake gently.

Mount in xylol balsam.

Other stains than boraxcarmin may be used, as may be seen by referring to the chapter on staining, or to the schemes following. If a large mass of tissue is to be hardened by corrosive sublimate, as a brain, it should remain in a saturated solution longer than the time here given, the time depending on the size of the organ. In such a case the fluid should be changed as it shows turbidity. Large tissues should be in the alcohol for a longer time than small ones.

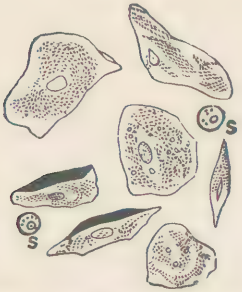
SCHEMES FOR SOME SPECIAL CASES.

Squamous Epithelium and Salivary Cells.— To obtain this, gently scrape the inner surface of the lip with the finger-nail or the section-lifter. Place the scrapings on a cover-glass, add a drop of saliva, and spread over the cover-glass. Skim off any air-bubbles with a needle. Dry the cover-glass and treat according to Scheme 4.

Fig. 5 shows something of what may be seen. The epithelium-cells will be seen as irregular-shaped flakes with the nuclei more deeply colored than the perinuclear parts. The salivary

cells may be seen less numerous than the others, as small round bodies, marked *S* in the figure.

FIG. 5.



Cells of squamous epithelium from the mouth: *S*, salivary cells or corpuscles.

Keep a frog in a small quantity of water for two days. Float on a cover-glass one or more of the cast flakes from the frog's mouth that may be seen on the surface of the water. Treat in the same way, but use hæmatoxylin instead of eosin. With (H.) the nuclei will be seen to contain several nucleoli.

SCHEME SIX.

Columnar Epithelium.—A convenient way to see this is to take the small intestine of a rabbit or a frog. Open the intestine and wash it out

with normal salt solution to remove the food particles. Then place the piece in—

Alcohol, 50 per cent., 24 hours.

Picrocarmin, 24 hours.

Scrape mucous coat and tease scrapings on centre of slide.

Add drop of formic glycerin.

Ring and mount.

It would be best to make the ring before putting the scrapings on the slide. Unless it is teased out well this will fail to show the separate columnar cells.

Instead of this formula, the fresh scrapings may be treated according to Scheme 4. If this is done, stain with eosin or safranine.

Ciliated Epithelium.—This is usually a form of columnar epithelium, though any form may be ciliated. To obtain this, gently scrape the roof of the mouth or the tongue of a frog and place the scrapings on a cover-glass. Treat this by Scheme 3. Methylene blue and safranine may be substituted for methyl green and eosin if desired.

Unless the cells are too much matted together, the separate cells should show cilia on one end.

They may sometimes be seen in the air-passages of the lungs.

The Tongue of a Frog for Ciliated Epithelium.—Fig. 6 shows what may be seen in a sec-

FIG. 6.

Ciliated epithelium of the tongue of a frog: *m*, muscular fibres. × 250.

tion of a frog's tongue where the epithelium is in form columnar and ciliated on the end. To prepare such a section, pin out the tongue on a thin section of cork, using thorns. Treat by Scheme 5. Under the layer of epithelium will be found the other tissues of the tongue.

SCHEME SEVEN.

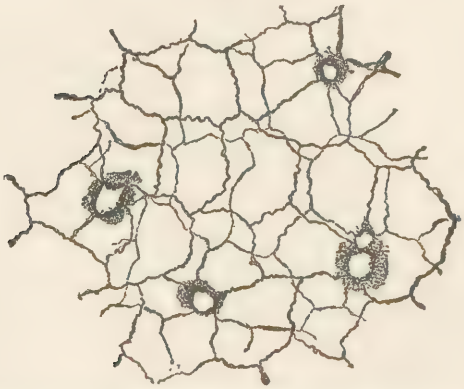
Endothelium.—This term is generally used to designate a thin layer of tissue that lines the interior of some organ, as the interior of a blood-vessel or the back part of the cornea.

In a broader sense it is used as a name for the outer layer of cells of the omentum or mesentery, the pleura, or any other organ that has no connection with the exterior part of the body. Fig. 7 shows such cells from the omentum of a rabbit, with some holes also. The method of preparation is as follows:

Wash to remove blood.

Pin on cork or fasten between rings.

FIG. 7.



Omentum of young rabbit stained with silver nitrate, showing endothelium on the upper and under surface, the outlines of the latter faintly indicated. × 300.

Place in silver nitrate, ½ per cent., till it is gray.

Place in water, exposed to light, till it is brown.

Alcohol, 70 per cent., a few hours.

Alcohol, 95 per cent., a few hours.

Alcohol, 100 per cent.

Clearing oil.

Mount in xylol balsam.

After silvering, part of the preparation may be stained with hæmatoxylin if desired.

SCHEME EIGHT.

Submaxillary Gland.—To see the real structure of this it should be double-stained by some one of the processes. Harden in alcohol by Scheme 1, following the scheme till the sections are cut. Then proceed as follows:

Aniline green, 5 minutes.

Wash rapidly in alcohol, 70 per cent. and 95 per cent.

Clear in clove oil in which a little eosin is dissolved.

Mount in xylol balsam.

Methylene blue may be used instead of aniline green. The mucous cells and ducts are green or blue, while the other parts are pinkish unless the washing is prolonged enough to wash out the green. Part of the nuclei should also be

dark. If Scheme 5 is used for the hardening, use methylene blue and safranine for stains.

STRUCTURE OF CELLS AND MITOSIS.

Physiology, as well as botany and zoology, teaches that living organisms are either a single cell or are built up of a multitude of cells. We cannot study the histology of any tissues without having to do more or less with cells. For this reason it seems best to present here something of the structure of cells.

The casual appearance of cells is shown to some extent in Figs. 3, 4, 5, 6, and 7. Ordinarily, cells are composed of two coats and fluid contents within. The outer coat, not always present, seems to be the inert part of the cell, the part that gives shape and character to some cells. The inner is protoplasmic, the active part when the cell is motile, or at least the part with which the motile filaments are connected. In animals it is not easy to see these parts, but they may readily be seen in some of the lower plants, as Spirogyra. In the centre or some part of the cell is the nucleus with one or more nucleoli. This is more or less readily detected in all living

cells, and seems to be essential to the life of the cell.

Plants and animals grow by a multiplication of the cells, and this takes place in the division of the cells. In this division it is the nucleus that divides first. Before dividing, the nucleus undergoes several changes. It is first round and of a granular form, then the granulations enlarge, gradually changing to a stellate form, and this finally dividing into two stars. At this stage the wall constricts, and the cell finally becomes two complete cells.

FIG. 8.

Epidermis of a young salamander. × 300.

The study of this is called mitosis. It is best seen in a young salamander or the tail of a tadpole that is growing rapidly, if studied in an animal. In plants, it may be seen in the developing part of a growing onion or in the developing ovary of such a plant as a lily. The characters of the cells are fairly shown in Fig. 8.

To get good results, harden for a week or ten days in $\frac{1}{6}$ per cent. solution of chromic acid. After this place in 35 per cent. alcohol, changing frequently till no color is shown in the alcohol. Pass from this up to 95 per cent. alcohol, where it should not remain more than a day or two before embedding.

If plant tissues are studied, iodine green is a good stain. Good results can be had by using any of the combinations of double stains. If animal tissues are used, safranine is a good stain. If it can be washed out till only the nuclei contain the stain, the cell markings will show best. To do this use acid alcohol for the first wash. Care will be required in the manipulation of this.

Muscles.—To see muscle-fibres, the transverse striæ, and the nuclei, treat by Scheme 1. Embed some so as to get transverse sections and others for longitudinal sections. Stain with boraxcarmin to show the muscle-fibres, or hæmatoxylin may be used, but should not be overstained. For nuclei and transverse striæ, double-stain with hæmatoxylin and boraxcarmin or hæmatoxylin and gold-orange. Picrocarmin may be

used for the same purpose. The double stain should show the sarcolemma also.

To separate muscle-fibres into disks, place small pieces for several hours in a saturated solution of ammonium carbonate. Tease out. This will show the sarcolemma, and inside this the muscle disks.

To get good results in staining for nuclei, take the muscles of a frog or some similar animal. Double-stained sections of the glandular stomach of a bird will show the nuclei of the involuntary muscles.

FIG. 9.

Muscle-fibre from rabbit: *a*, dim disk; *b*, light disk; *c*, intermediate line; *n*, nucleus seen in profile. × 300.

Fig. 9 shows a muscle from a rabbit where both muscle disks or striæ and nuclei are shown.

Generally the striæ will be seen as fine lines across the muscle-fibre with (H.). Sometimes wavy transverse marks will be seen with (L.) in some of the involuntary muscles, as the gizzard of a bird. This kind of marking should not be mistaken for the transverse striæ of the voluntary muscles, for they cannot be seen with low power.

SCHEME NINE.

Bones.—Before bones can be cut with the microtome they must be decalcified or the lime taken out of them. After most of the decalcifying fluids have been used, the tissues need to be hardened in alcohol. Picric acid, however, is both a hardening and decalcifying fluid. Only small bones should be used, and these cut into short pieces. Remove the flesh but leave the periosteum. The scheme is:

Picric acid, saturated, 2 weeks or longer if not decalcified.

Wash in 50 per cent. alcohol.

Alcohol, 70 per cent.

Alcohol, 95 per cent.

Thin celloidin, 24 hours.

Thick celloidin, 24 hours.

Embed and put in 70 per cent. alcohol for 24 hours.

Cut, putting sections in 70 per cent. alcohol.

Picrocarmin, 1 to 3 minutes.

Acid alcohol + picric acid.

Alcohol, 70 per cent., + picric acid.

Alcohol, 95 per cent., + picric acid.

Clearing oil + picric acid.

Mount in xylol balsam or Farrant's medium.

But a small amount of the picric acid should be used, just enough to be dissolved. If the Farrant medium is used it should be ringed afterwards, as this does not dry hard.

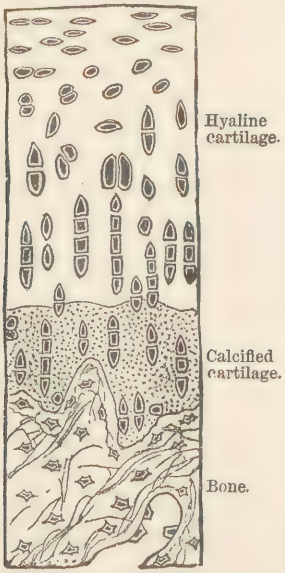
Embed in such a way as to get both transverse and longitudinal sections from two pieces. This way will show the canals in the bones to the best advantage.

If it is desired to get sections of a vertebra, Scheme 14 will be preferable to this, as the chromic acid is better to fix the nerve-elements in the spinal cord.

If the end of a bone is used, the interarticular cartilage will be shown in a longitudinal section. Fig. 10 shows this, giving a very good view of the two tissues and of a tissue that is part bone

and part cartilage, the "calcified cartilage." This is from a section hardened in chromic acid and stained with picrocarmin.

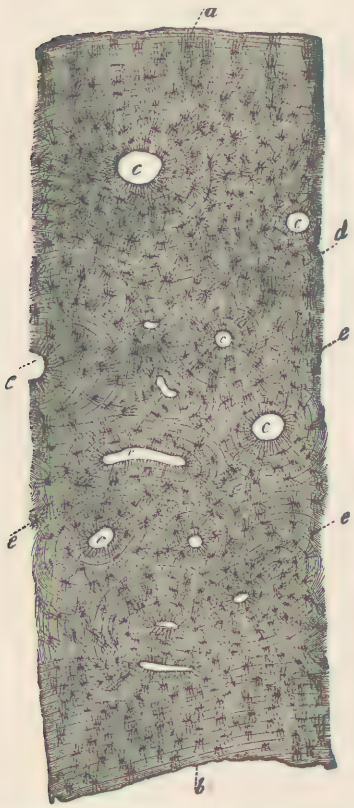
FIG. 10.



Articular cartilage and bone.

Fig. 11 represents a cross-section of a human metacarpal bone. It may not be possible to see all these parts in a decalcified section, as this cut is from a ground section, but most of them can

FIG. 11.



Human metacarpal bone: *a*, peripheric lamellæ; *b*, perimedullary lamellæ; *c*, Haversian canals surrounded by their Haversian lamellæ; *e*, lacunæ. × 20.

be seen in a carefully prepared softened section. The explanations of the figure give the parts.

SCHEME TEN.

Intervertebral Disk.—To prepare this, take two connected vertebræ of some small animal, as a rat, small cat, or rabbit, cut off as much of the flesh as possible and place the piece in—

Picrosulphuric acid, 24 hours.

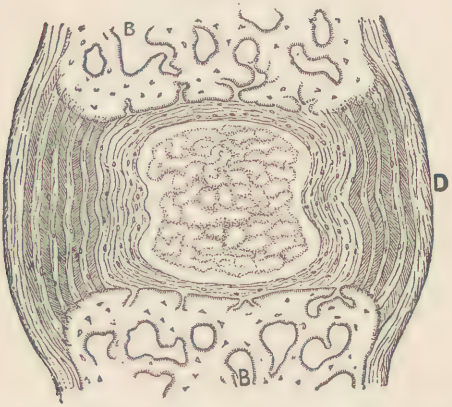
Wash thoroughly in water.

Harden in alcohol, 50 per cent., 70 per cent., and 95 per cent., 24 hours each. After this treat by Scheme 9, beginning with "thin celloidin." Instead of using this scheme the specimen may be prepared by Scheme 14, which may, perhaps, be the best. In either case picrocarmin will be the most appropriate stain.

Fig. 12 shows the structure of a section of such a disk. The upper and lower parts of the figure represent the ends of the vertebræ, the middle the pulp of the disk that is regarded as a remnant of the "chorda dorsalis." Next to *D* is shown the ligament, and between this and the central pulp the bundles of fibro-cartilage. The little dots are the nuclei in the cartilage.

Red Marrow of Bones.—Interesting studies may be made of marrow. Especially will this be the case if the red marrow be used, for some think that some of the cells to be found in red

FIG. 12.



Intervertebral disk of a cat: *B*, bone; *D*, disk. Prepared by chromo-nitric fluid. × 15.

marrow are the source of the red corpuscles of the blood. Squeeze out the red marrow from the cut end of a rib of a rat and diffuse it over a cover-glass. Dry, after which, set by passing several times through the flame of a spirit lamp or a Bunsen burner. Treat after this by Scheme 3. For stains, eosin-methylene blue or

Ehrlich-Biondi-Heidenhain may be used instead of those mentioned in the scheme. This form of marrow is to be found in the heads of the long bones, and a study of it may enable the student to better understand what he sees in a section of this part of a bone.

Kidney.—To make sections of the kidney, treat portions of this organ by Scheme 1 or Scheme 5, the latter being the best. To get the most out of the study of the kidney, several sections should be differently stained. Borax-carmin will give the general structure. For seeing the nuclei of the cells, use some of the double stains,—hæmatoxylin and gold-orange, hæmatoxylin and boraxcarmin, or Ehrlich-Biondi. The latter will show the capsule to the best advantage.

The kidney is composed of a series of round bodies, the Malpighian bodies, which are the real excreting parts of the organ. Outside of these and leading from them to the pelvis of the kidney are the tubules, not straight but variously bent. One of these Malpighian bodies is shown in the centre of Fig. 13, with the tubules round it. The dots in the cells of the

tubules are the nuclei of the cells just as they may be seen in a double-stained section. The Malpighian bodies are in the outer part of the

FIG. 13.

Glomerulus, or tuft of capillaries round a Malpighian body of kidney, and sections of convoluted tubules.

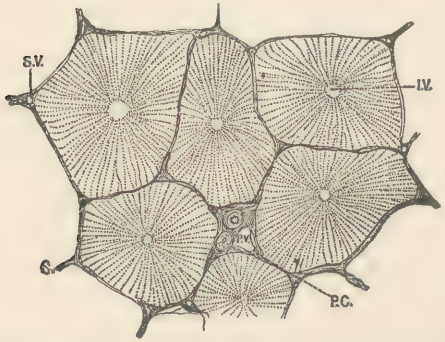
kidney, while the tubules are gathered in the interior into bundles or pyramids, not opening into the pelvis except at the apices of these pyramids. To double-stain, study carefully the chapter on double-staining.

Liver.—To make sections of this organ the same directions may be followed as are given for the kidney. The pieces may be treated by

Scheme 1 or Scheme 5. The same directions for staining may also be followed.

The liver is composed of lobules about $\frac{1}{20}$ of an inch in diameter. Its outside is covered with a serous membrane, the capsule. The lobules are made up of very small cells, polygonal in shape and about $\frac{1}{1200}$ of an inch in diameter, each with one or two nuclei. These may be seen with (H.) in a double-stained section.

FIG. 14.



Liver of a pig, showing lobules: *P.C.*, portal canal containing bile-duct, hepatic artery, and portal vein (*P.V.*); *S.*, septa; *S.V.*, sublobular vein; *I.V.*, intralobular vein.

The lobules are surrounded by a more or less distinct layer of connective tissue and the interlobular veins, branches of the portal vein.

These send in a plexus of veins into the interior of the lobule, supplying each cell with blood. The blood is gathered into another plexus, which finally empties into the centre of the lobule, the intralobular vein, a branch of the hepatic vein. Fig. 14 shows several lobules of the liver of a pig. This may be regarded as partly diagrammatic, as only the liver of a few animals show the parts as well as these are shown. Besides the portal vein, the liver is nourished by its own artery, the hepatic artery.

Pancreas.—Scheme 5 is perhaps the best method to treat this organ. The real structure of the parts will be brought out by using picrocarmin or some other double stain. Ehrlich-Biondi stain will bring out the capsule and the trabeculæ running into the interior from this part as well as the structure of the tubules.

The pancreas is a gland that may be said to be composed of compound tubules, several of these opening into one duct. The structure of these tubules is seen in Fig. 15, from the pancreas of a dog. Any of the double stains should show the gland-cells. As seen here, these are

somewhat columnar with the nuclei near the base.

Fig. 15.

Pancreas of a dog: *A*, ascinus; *C*, capsule; *D*, duct. Prepared by corrosive sublimate and stained with picrocarmin. × 300.

Spleen.—Treatment of this organ by Scheme 5 will produce good results, though Scheme 1 may be used. Besides boraxcarmin, the double stains may be used. To show the trabeculæ and capsule, use the Ehrlich-Biondi stain. This will also differentiate the blood-vessel walls.

The spleen is a dark-red ductless gland whose use is not well understood. It is thought to share with the red marrow of the bones in the development of the blood-corpuscles, while some think that it produces the white corpuscles or

leucocytes and destroys the red corpuscles. It is composed of a covering outside, or capsule, which sends fibres or trabeculæ into the interior,

FIG. 16.

T. S. of part of human spleen. × 10.

and the net-work of blood-vessels. Scattered through the organ are round bodies, the Malpighian bodies of the spleen, and the rest of the organ is made up of the spleen pulp. This con-

sists of a mesh of branched cells with membranous expansions which anastomose with the neighboring cells. This net-work is permeated by the blood-corpuscles. Fig. 16 shows a section of the human spleen.

Soft Palate for Mucous Cells.—If it is desirable to make a study of mucous cells, take the soft palate of a rabbit or a dog, harden and prepare for staining by Scheme 5. For stains use aniline green and eosin. The last may be applied by dissolving in the clearing oil, but care should be taken to have a complete solution of the eosin. Treated in this way the epithelium and connective-tissue cells will be red, with their nuclei blue, and the mucous glands will be bluish.

Skin.—For a simple study of the skin, the ear of a rat or other small animal may be hardened and prepared by Scheme 1. This may be stained with boraxcarmin or double-stained, using eosin-methylene blue or any other of the combinations. The first will show the hairs and hair-follicles and the two layers of the skin. If double-stained, these will be better seen, and with (II.) the cells of the parts of the skin and

the glands can be studied. The cartilage will be seen in the centre as a line of large cells.

Pieces of the skin of any animal may be taken instead of the ear, as suggested above.

SCHEME ELEVEN.

Stomach.—Parts should be taken from the cardiac and from the pyloric ends of the stomach. If necessary, pin these out flat before hardening. Prepare by Scheme 1 or Scheme 5 or by the following:

Müller's fluid, 5 to 7 weeks.

Thoroughly wash in water.

Alcohol, 50 per cent., 70 per cent., and 95 per cent., 24 hours each, in the dark.

Thin celloidin, 24 hours.

Thick celloidin, 24 hours.

Embed, putting in 70 per cent. alcohol, 24 hours.

Cut, putting sections in 70 per cent. alcohol.

Indigocarmin till bright blue or red-blue.

Oxalic acid, saturated, ½ hour.

Alcohol, 50 per cent.

Alcohol, 70 per cent.

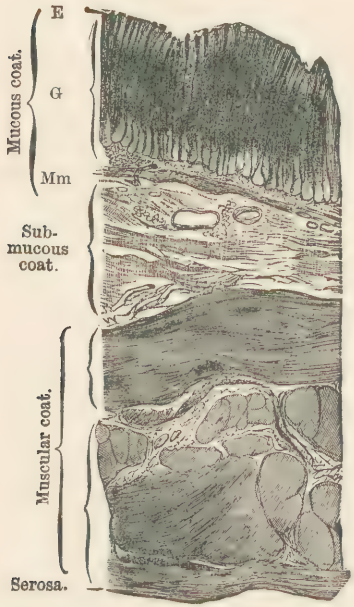
Alcohol, 95 per cent.

Clearing oil.

Mount in xylol balsam.

In the cardiac end of the stomach the glands

FIG. 17.



V. S. of wall of human stomach: E, epithelium; G, glands; Mm, muscularis mucosæ. × 15.

are simple tubes, but in the pyloric end they are branched and the opening to the tube is larger. Fig. 17 shows a section of the human

stomach from the cardiac end. The only advantage in using Müller's fluid for the hardening is that the cells of the glands will retain more of their original shape. Very good results may be had by using alcohol, or corrosive sublimate and alcohol, for hardening.

As shown in the figure, the stomach has four coats or layers. The inner is the mucous coat, and contains the glands for secreting the gastric juice, or the peptic glands, as they are called.

In the intervals between digestion the stomach secretes mucus, but after food passes into the stomach the peptic glands secrete the gastric juice. The submucous coat is mostly connective tissue, forming a support for the blood-vessels and nerves of this organ. The third coat contains the muscles, usually described as in three layers. The outer coat is a thin layer of serous membrane that can readily be seen by proper staining.

Glandular Stomach of a Bird.—In birds the glandular stomach and the gizzard correspond to the stomach in mammals. For sections of the glandular stomach take any of the smaller birds, as the English sparrow, blue jay, or

meadow-lark. The chicken has this part so large that it is not suitable for good sections, and the plumose glands are set more obliquely in the organ than in the smaller birds.

FIG. 18.

V. S. of mucous membrane of the stomach of a cat.

Treat the specimen by Scheme 1 or Scheme 2. Boraxcarmin is a good stain for showing the general structure of this organ. To differ-

entiate the tissues double-stain with picrocarmin, hæmatoxylin and boraxcarmin, or Ehrlich-Biondi.

The glandular stomach of a bird, located in front of the gizzard, is the secreting organ for the digestion of the food, as but little secreting, if any, can be done by the gizzard. The inner layer of the mucous coat is a series of straight glands. The cells are columnar, the nuclei near the base, and the outer end of the cell bends a little outward. These glands are tubes, the outer end opening into the interior of the organ. The walls of these tubes are areolar connective tissue, as may be seen by using Ehrlich-Biondi or hæmatoxylin and boraxcarmin stains. Outside these are a series of large plumose glands that are separated from each other by areolar connective tissue. The duct of each gland is in its centre, and opens into the interior of the organ between the tubular glands. These glands are evidently peptic. The cells are short columnar, the nuclei are about in the centre of the cells. Fig. 19 shows these parts as seen by low power.

Outside the plumose glands there is a very

thin submucous coat, not shown in the figure, which is followed by the two layers of the muscular coat and the thin serous membrane.

FIG. 19.

The glandular stomach of a meadow-lark. The outside shows the muscular and serous coats combined, inside this the large plumose glands, and near the centre the tubular glands. × 12.

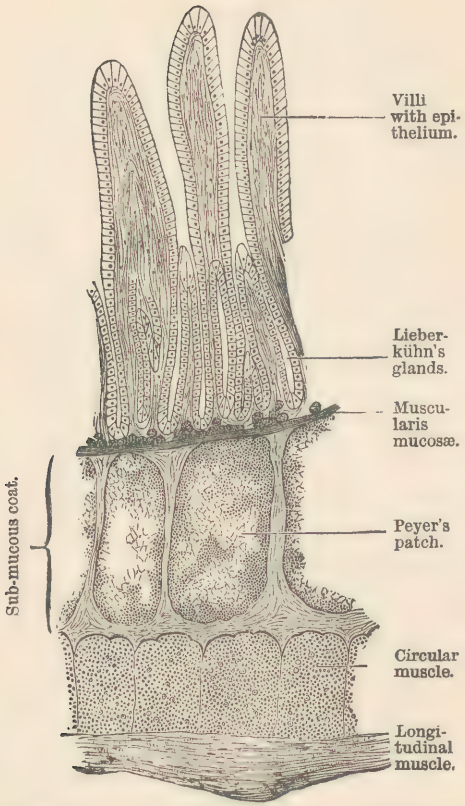
Gizzard.—The gizzard in a bird is essentially a masticating organ. The two outer coats correspond to the two outer coats of the human

stomach, serous and muscular. On account of the extra work that is put on this organ the muscular coat is unusually thick. Inside this is a coat that seems to be wholly fibrous. In the grain-eating birds the inner part of this is compact, so that it is not injured by the sharp points of gravel, etc. In the Raptores the whole of this membrane is fibrous, as the outer part of the membrane is in grain-eating birds. Treat the same as the glandular stomach and use the same stains.

Small Intestines.—For a study of this take small pieces of the duodenum, jejunum, and ileum of a cat, rat, or other small animal. Very good results may be had by treating these by Scheme 1 or Scheme 2. To see the gland-tissues to the best advantage, the hardening may be done by Scheme 11. In this case stain with picrocarmin.

Fig. 20 shows the structure of a section of the small intestine of a dog. It consists of four coats similar to those of the stomach. The inner or mucous coat contains first the villi and below these the follicles or glands of Lieberkühn. If the section be from a cat or a dog, the Peyer's

FIG. 20.



L. S. through the Peyer's patches of the small intestine of a dog.

patches or glands may be looked for in the outer part of the mucous coat, as seen in the figure.

These are mostly separated from the follicles of Lieberkühn by a thin layer of smooth muscular tissue, but some of them will show a pointed end protruding through this layer. A double-stained section will show the structure of all these parts. In the first part of the duodenum look for the racemose glands of Bruner in the submucous coat.

The submucous coat is connective tissue. Outside this is the muscular coat in two layers. In a cross-section the longitudinal muscles will show their cut ends. The outer coat is a thin layer of cells, the serous coat.

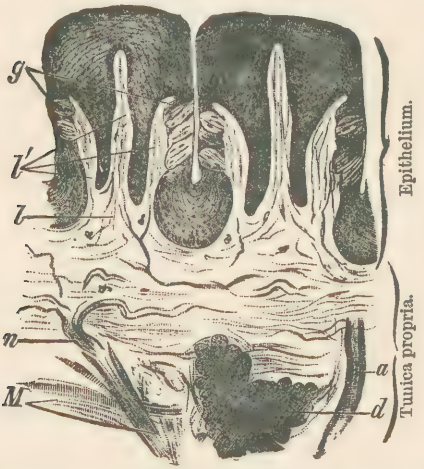
The Peyer's patches are found only on one side of the intestine, the side to which the omentum is attached.

Tongue.—Cut the tongue of a cat or a rat in such a way that sections can be had of the tip and base. The preparation may be by Scheme 1 or by Scheme 5. Make some of the sections longitudinal and some transverse. For the ordinary structure, stain with boraxcarmin. Double stain some with methyl-green and eosin, and others with eosin-methylene blue. In the first the connective tissue and papillæ are red-

dish, the taste glands or buds reddish, but the mucous glands will be bluish.

The tongue has on its upper surface several forms of papillæ, a row in the form of a V across the back part being called the circumval-

Fig. 21.



Foliate papillæ from a rabbit: *l*, *l'*, primary and secondary septa; *g*, taste buds; *n*, nerve; *d*, serous gland; *a*, its duct; *M*, muscular fibres. × 80.

late. Each of these is surrounded by a depression or fossa, and in this fossa are to be found the taste buds in the substance of the papilla. Fig. 21 shows a section from the tongue of a rabbit

where two papillæ are seen with taste pits or buds in their sides. In this case the two papillæ are close together, with the fossa or opening between them.

SCHEME TWELVE.

Trachea and Lungs.—Take two pieces of the trachea of a cat or a rat and of a chicken, one for transverse and the other for longitudinal sections. They may be prepared by Scheme 1 or by Scheme 5 and stained with boraxcarmin or hæmatoxylin or picrocarmin. The best preparations may be made as follows:

Trachea in chromic acid, .2 per cent. solution, 10 days.

Wash thoroughly in water.

Alcohol, 50 per cent., 24 hours.

Alcohol, 70 per cent., 24 hours.

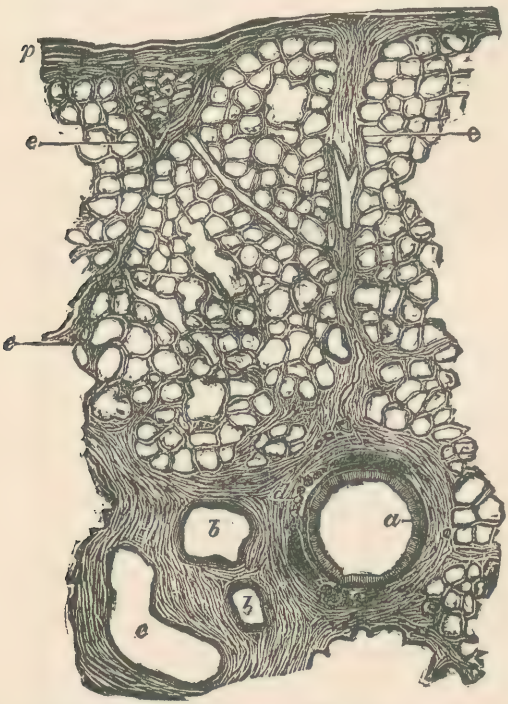
Alcohol, 95 per cent., 24 hours.

For the rest treat by Scheme 9, beginning at "thin celloidin."

Keep from the light during the process of hardening. Treat the pieces of the lung in the same way as the last, or they may be treated by Scheme 1 or Scheme 5. If it is desirable to show the structure of the lung, such as the

nuclei of the cells, etc., double-stain or stain with Ehrlich-Biondi.

FIG. 22.



V. S. of human lung: *p*, pleura; *a*, epithelium of a bronchus; *b*, blood-vessel; *c*, pulmonary vein; *d*, cartilage; *e*, interlobular septum, continuous with the inner layer of the pleura. × 25.

Fig. 22 shows the structure of the human lung. The lung of a cat will show the same

general characters, and that of a chicken will differ but little. In the lung of a frog the cells are much larger. In the human trachea and in the trachea of a cat the cartilaginous rings are not entire, but have an opening on the back side that is filled out with other tissue. In the trachea of a chicken the rings are entire and overlap each longitudinally, as a longitudinal section will show.

FIG. 23.

Nerve-fibre: *a*, axis cylinder; *b*, Ranvier's node; *c*, nucleus. ×200.

SCHEME THIRTEEN.

Nerve, showing Axis Cylinder.—For this take any small nerve, the smaller the better. Treat as follows:

Bichromate of potash, 2 or 3 days. (Use a .2 per cent. solution.)

Thoroughly wash in water.

Alcohol, 50 per cent., 70 per cent., 95 per cent., 12 to 24 hours each.

Boraxcarmin.

Dehydrate and put in clearing oil.

Tease out and mount in xylol balsam.

Fig. 23 shows a nerve that has been treated in this way. The axis cylinder should be more deeply stained than the myelin.

SCHEME FOURTEEN.

Spinal Cord and Brain.—This may be prepared in several ways, by Scheme 1 or Scheme 5, and get very good results, but the best will be when some of the chromic acid compounds are used. For this purpose treat by Scheme 11 till the sections are ready to stain. Then instead of the stain given there use the following:

Ehrlich-Biondi fluid, 4 to 6 hours, or heat till vapor begins to rise.

Wash quickly in 95 per cent. alcohol.

Alcohol, 100 per cent.

Clearing oil.

Mount in xylol balsam.

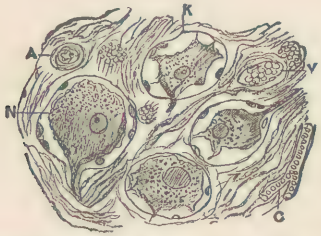
While staining watch it that the sections do not stain too deeply. Watch the sections also while in the clearing oil that too much of the stain does not wash out. For the reason that this stain is apt to wash out, the sections should not remain in the clearing oil. If the stain is

just right from the 100 per cent. alcohol, xylol may be used for a clearing oil.

Picrocarmin makes a good stain for either of the schemes instead of those given. The nuclei of the cells may be seen well with this or with a double stain of boraxcarmin and hæmatoxylin.

Fig. 24 shows a few nerve-cells from one of the sympathetic ganglia. The nerve-cells in the

FIG. 24.



Human sympathetic ganglion: *A*, small artery; *C*, capillary; *V*, vein; *K*, capsule; *N*, nerve-cells. × 300.

brain and spinal cord will be similar to these, but may not all be of the same shape. For the structure of these parts see the physiology.

The Eye.—Make a single small cut with a razor at the equator of the eyeball of a cat or a rabbit, and place the eye for 24 hours in a .25 per cent. solution of chromic acid. Then cut the eye into an anterior and posterior part

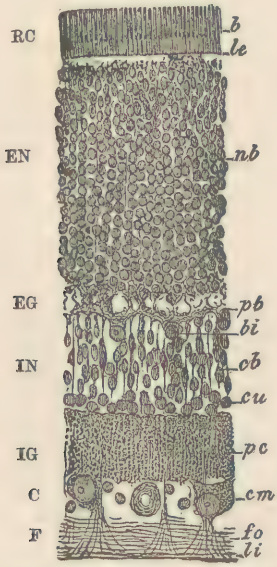
and place them for several days in the same fluid. Wash in water and finish the hardening in 50 per cent., 70 per cent., and 95 per cent. alcohol, 24 hours each. Finish by Scheme 9, beginning at thin celloidin. Stain most of the sections in picrocarmin and boraxcarmin and some with hæmatoxylin-boraxcarmin.

The anterior part of the eye will show the cornea, iris, lens, and the three coats of the eye back of the lens, though not so well as the posterior part. The cornea will show on its front the mucous membrane, or conjunctiva, and on its back a layer of endothelium cells. The lens should show something of its structure and of the membrane enclosing it. At the ends of the lens as it appears in the section may be seen the suspensory ligament, ciliary process, the ciliary muscles, and the parts of the iris. The double-stained sections should show the cell structure of these parts.

The posterior part of the eye will show the three coats, and should show the layers of the retina. Fig. 25 is from the retina of a cat. The sections are usually too thick to show the individual cells as plainly as seen in this, but

the different parts can be made out in a good section.

FIG. 25.



V. S. of retina of cat: *b*, rods and cones; *le*, external limiting membrane; *li*, internal limiting; *nb*, nuclei of rods; *pb*, basal plexus; *cb*, bipolar cells; *cu*, unipolar cells; *bi*, internal basal cells; *pc*, cerebral plexus; *cm*, multiple nerve-cells; *fo*, fibres of optic nerve. The initials on the left are those of the usual names,—rods and cones, external nuclear, external granular, internal nuclear, internal granular, cellular, and fibrous.

If the eye of a chicken is used, sections through the region of the optic nerve should show the marsupium. This is a membrane that extends from the optic nerve to or towards the

lens. It stands vertical in the eye, hence if the eye is embedded so as to cut sections from the bill towards the back of the head, as the eye is in the head, the sections will show the marsupium as a line. Mammals do not have this.

SCHEME FIFTEEN.

Tooth.—For sections of this take the lower jaw of a cat or a rat, cut it into short pieces, and treat as follows:

Chromic acid, .2 per cent. solution, 2 or 3 days.

Chromic nitric acid fluid, 2 or 3 weeks.

Thoroughly wash in water.

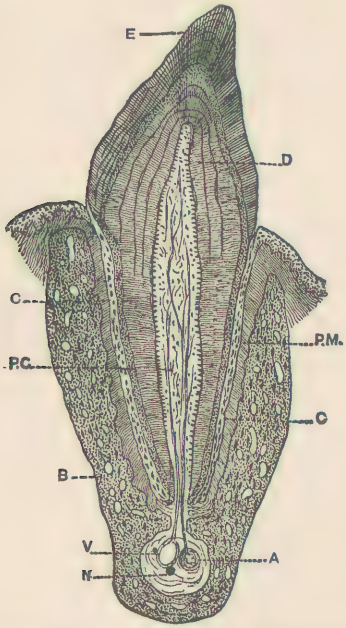
Alcohol, 70 per cent., 24 hours, in the dark.

Alcohol, 95 per cent., 24 hours, in the dark.

Embed and cut as per Scheme 1, using picrocarmin as a stain. Fig. 26 shows a section of a human tooth in the bone. A section of the tooth of a cat will show essentially the same structure, but whether it shows all these parts as here shown will depend upon whether it is through the centre of the tooth. A section of a cat's tooth will usually show the alveolar process more distinctly than is shown in the figure, the parts of the tooth, the enamel and dentine

outside the jaw, and the cement and dentine in the jaw. There will be some muscular tissue adherent to the jaw that may be seen and recognized.

FIG. 26.



V. S. of human tooth in jaw: *E*, enamel; *D*, dentine; *P.M.*, periodental membrane; *P.C.*, pulp-cavity; *C*, cement; *B*, bone of jaw; *V*, vein; *A*, artery; *N*, nerve.

Any bone-tissue may be treated by this scheme and stained with picrocarmin. Keep in the acid till a pin can be pushed into the tooth.

INDEX.

THE END.